THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 107 FEBRUARY 1, 1934 No. 2

THE EFFECT OF HEMORRHAGE ON THE NORMAL AND ADRENALECTOMIZED DOG[1,2]

W. W. SWINGLE, J. J. PFIFFNER, H. M. VARS AND W. M. PARKINS

From the Biological Laboratory, Princeton University

Received for publication October 3, 1933

In a recent preliminary publication (1933) the writers stated that when the adrenalectomized dog with well healed wounds, free from infection of any kind, and enjoying normal health, is taken off extract, the blood and plasma volume fall to a low level owing to loss of fluid from the circulation. So far as we have been able to determine, the material lost probably consists only of fluid and (as shown recently by Loeb et al., 1933, and Harrop et al., 1933) salts, there being little if any loss of red cells or of plasma protein; although we have no actual data on this latter point.

The decrease in circulating volume of blood results in a progressive decline in arterial pressure which eventually reaches such low levels as to be incompatible with life. Hemoconcentration invariably occurs as a consequence of the diminution in the fluid portion of the blood, leading to increases in hemoglobin, plasma proteins and blood solids. Numerous investigators have called attention to the marked blood concentration which appears following adrenal extirpation (Gradinescu, 1913; Kellaway and Cowell, 1923; Lucas, 1926; Rogoff and Stewart, 1926; Viale and Bruno, 1927; Swingle, 1927; Harrop and Weinstein, 1933, and others). However, in this communication we are not primarily concerned with this phase of the work, and will return to a discussion of blood concentration and blood volume in a later paper.

[1] In the collaborative studies on the adrenal cortex carried on in this laboratory the writers have specialized in certain phases of the experiments. The physiological aspect of the problem is the responsibility of the senior author (W. W. S.); the biochemical work of extraction and purification of the hormone that of one of us (J. J. P.).

[2] These investigations have been aided by a grant from the Josiah Macy Jr. Foundation, New York.

Preliminary experiments performed in this laboratory indicated that the dog lacking adrenals also lacks a vital part of its mechanism for blood dilution. Without the normal functioning of the mechanism whereby dilution occurs, hemorrhage of almost any degree should be likely to prove serious. It was with this idea in mind that the present study was undertaken.

Most of the animals, both normal and adrenalectomized, were bled from the femoral artery. Several dogs were bled directly from the left heart. In two cases the blood was removed from the venous side of the circulation by way of the jugular vein (protocol 11). In those experiments on normal dogs where the hemorrhage involved loss of several hundred cubic centimeters of blood, a local anesthetic was used at the site of cannulation, the femoral artery exposed and cannulated with a paraffin coated glass cannula. The blood was taken from the adrenalectomized dogs in syringes and no anesthetic was employed.

In presenting the data the following abbreviations are used. Bp. is mean arterial pressure in millimeters Hg, as measured directly in the femoral artery by the method of Dameshek and Loman (1932), Parkins (in press); Hb. is hemoglobin in grams per cent as determined by the Newcomer method; urea is blood urea nitrogen in milligrams per cent, determined by the gasometric (hypobromite) method of Van Slyke; blood glucose when given, is in milligrams per cent, and was obtained on tungstic acid filtrates with Somogyi modification of the Shaffer-Hartman reagent. Except when stated to the contrary the blood was taken from the femoral artery.

Normal dogs vary as to their degree of resistance to hemorrhage (Gesell, 1918; Meek and Eyster, 1920; Carrier, Lee and Whipple, 1922; Blalock, 1927; Adolph, Gerbasi and Lepore, 1933; and others). Blalock (1927) states that shock symptoms are first noted when from 20 to 30 cc. of blood per kilogram have been removed. According to his data, withdrawal of amounts in excess of 40 cc. per kilogram generally caused the death of the animal.

It requires the loss of relatively huge amounts to significantly reduce the mean arterial pressure. For example, a dog weighing 10 kgm. which exhibits a decline of only 10 mm. of Hg in arterial pressure after losing 300 cc. may show a decline of an additional 40 mm. of Hg after losing 30 cc. more blood. Blalock (1927) has called attention to the fact that such observations accord with Porter's (1925) conception of a critical level of blood pressure, and indicates that 30 cc. of blood per kilogram is for most dogs, approximately the critical amount of blood which can be taken without inducing severe shock.

Porter (1925, and earlier papers) described what he termed the critical level of blood pressure. When the pressure is above the critical level,

large quantities of blood can be taken without significantly altering the animal's condition. However, when the pressure stands at the critical level, the loss of 50 cc. may be fatal. According to Porter, if the arterial pressure is at the critical level, the arteries are partly empty, and "to empty them still further may be mortal."

The writers wish to emphasize this so called critical level of blood pressure in the dog, since the hemorrhage experiments on the adrenalectomized animals were performed when the mean arterial pressure was at a level varying (in the different cases) between 92–74 mm. of Hg.

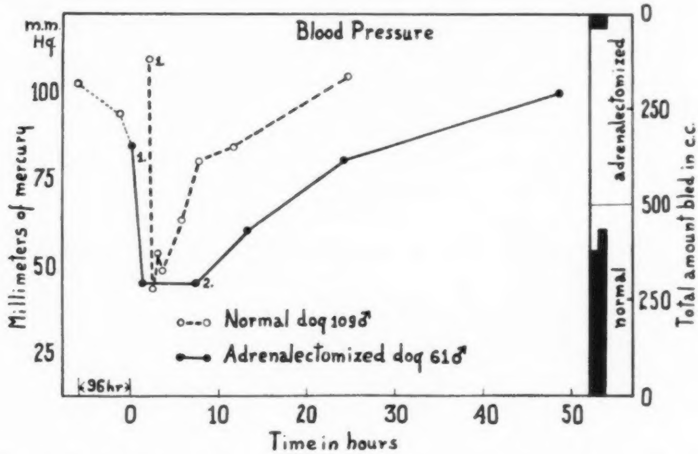


Fig. 1. Comparison of the effect of hemorrhage in the normal and adrenalectomized dog upon the mean arterial pressure. The last injection of hormone was given 96 hours previous to bleeding. At *1* the blood was removed, and at *2* the cortical hormone was injected. The normal dog was bled 47 cc. per kgm.; the adrenalectomized dog, 4 cc. per kgm. Detailed data are given in protocols 1 and 6.

Normal unanesthetized dogs with intact adrenals can withstand huge blood losses without serious consequences. We have bled animals to the extent of removing 47 to 54 cc. of blood per kilogram (protocols 1 and 2) and observed that within 15 to 20 hours afterwards the animals had diluted and returned to normal, in so far as blood pressure, vigor and appetite were concerned. The dogs replenish their depleted blood volumes by rapid withdrawal of fluid from the tissues into the blood stream, and by drinking water. The abbreviated protocols of a few typical cases are cited below. The results of a typical case are graphically represented in figure 1.

Protocol 1. Dog 109. Normal male. Date 2/22/33. Weight 9.1 kgm. Animal untrained for blood pressure work. 11:30 a.m. pulse 80, Bp. 128. From 11:40–11:45 drew 365 cc. blood. At 11:50 a.m. pulse 128, Bp. 38–41. Animal prostrate and in

severe shock. 12:10 p.m. pulse 120, Bp. 53. At 12:20 removed 28 cc. Hb. 14.3. At 12:30 removed 35 cc. additional making a total of 428 cc. blood taken within 50 minutes or 47 cc. per kgm. 12:35 p.m. pulse 160, Bp. 47. At 1:40 dog drank 230 cc. water. 2:40 pulse 168, Bp. 63. Dog much improved. 5:00 p.m. pulse 124, Bp. 80. At 9:00 p.m. pulse 100, Bp. 83, Hb. 9.3. Animal left in cage over night with plenty water to drink. Following morning 2/23/33 at 10:10 a.m. pulse 84, Bp. 104, Hb. 8.2. Dog had taken 630 cc. water since 9:00 p.m. previous evening. Animal appeared normal, ate usual ration and was active and vigorous. Experiment was discontinued.

It will be noted that this animal drank a total of 860 cc. of water during the course of the experiment. For sake of comparison with similar experiments performed on adrenalectomized animals, attention is called to the fact that more blood was withdrawn from the normal dog after the shock level of 40 mm. Hg Bp. was reached without death following, than we were able to take from an adrenalectomized dog, seemingly in normal health with a Bp. of 84. (Figure 1, Dog 61.)

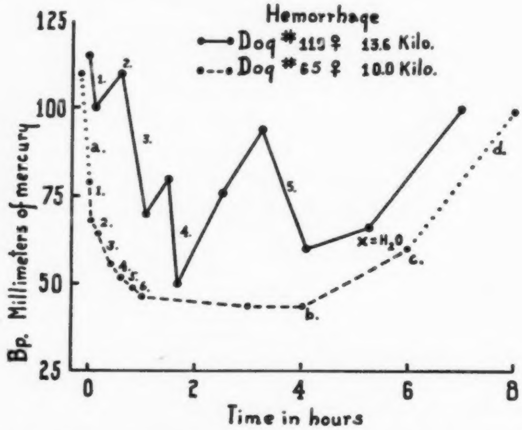


Fig. 2. The effect of hemorrhage in graded amounts upon the mean arterial pressure of the normal and adrenalectomized dog. Normal dog. *1* = 25 cc. of blood per kgm.; *2* = 35 cc. per kgm.; *3* = 45 cc.; *4* = 50 cc. and *5* = 54 cc. per kgm. At *X* the animal was permitted to drink water. Detailed data are given in protocol 2. Adrelanectomized dog. *a* = Interval between withdrawal of extract and bleeding (four days). *1* = 20 cc.; *2* = 5 cc.; *3* = 15 cc.; *4* = 7 cc.; *5* = 10 cc.; *6* = 6 cc.; (6.3 cc. per kgm. total) blood withdrawal. *b* = extract injected; *c–d* = 18 hour interval for this dog.

Protocol 2 illustrates the marked effect of fluid intake by mouth upon the Bp. of the dog in shock, when the fluid reservoirs of the tissues are inadequate to meet the deficit caused by massive hemorrhage.

Protocol 2. Dog 119. Normal female. Weight 13.6 kgm. 3/17/33 at 10:45 a.m. pulse 82, Bp. 115. At 10:50 withdrew 340 cc. blood or 25 cc. kgm. 11:00 a.m. pulse

110, Bp. 100. At 11:25 Bp. 110. At 11:30 drew 136 cc. blood or 35 cc. per kgm., pulse 88, Bp. 110. At 11:50 drew additional 136 cc. or total of 612 cc. or 45 cc. per kgm., pulse 200, Bp. 70. At 12:15 p.m. pulse 200, Bp. 80. At 12:20 drew 68 cc. or 50 cc. blood per kgm., pulse 218, Bp. 50. Animal in shock. Walks with difficulty. At 1:15 p.m. dog appears much brighter and stronger. Walks about. Bp. 76. At 2:00 p.m. Bp. 94. Between 2:30 and 2:45 p.m. removed an additional 54 cc. blood making a total bleeding of 734 cc. or 54 cc. per kgm. At 2:50 p.m. pulse 220, Bp. 60. Animal very weak 4:00 p.m. pulse 200, Bp. 67. Animal drank 800 cc. water by 3:30 p.m. At 5:45 p.m. pulse 168, Bp. 98. Animal appeared strong and vigorous. Experiment discontinued. The data are graphically shown in figure 2.

The following two protocols illustrate the effect of withholding and of administering water when the animal has been bled to the point where symptoms of profound shock appear. Protocol 3 is that of an animal which died from hemorrhagic shock because of water deprivation; and protocol 4 shows the striking beneficial effect of fluids upon such animals. Where the bleeding is of great magnitude and deep shock is induced, complete recovery and return of the animal to normal condition is not possible unless adequate fluid intake is permitted. This is to be expected, since under ordinary conditions, the tissues and interstitial fluid reservoirs of the normal animal are not sufficient to make up for the quantity of fluid lost by hemorrhage.

Protocol 3. Dog 134. Normal male. Weight 10.8 kgm. 5/2/33. At 11:30 a.m. pulse 100, Bp. 156 (animal untrained for Bp. work). 11:35–11:37 withdrew 270 cc. blood or 25 cc. per kgm. 11:38 Bp. 50. At 11:40 Bp. 63, at 11:48 Bp. 95, at 12:00 m. Bp. 133, at 12:10 p.m. Bp. 138. Dog normal in all respects. 12:12 p.m. drew 54 cc. blood or total 30 cc. per kgm. or 324 cc. 12:17 Bp. 100, pulse 144. At 12:25 removed 108 cc. blood. 12:30 Bp. 30–35 animal in deep shock. At 1:45 animal very weak. Can walk but sways about unsteadily. 2:35 p.m. pulse 190, Bp. 70. At 4:00 p.m. pulse 200, Bp. 88. Animal much stronger. Walks about in excellent shape. 4:05 drew 18 cc. blood. 4:10 Bp. 62; drew 10 cc. blood at 4:15, Bp. 56; drew 7 cc. at 4:20, Bp. 50, pulse 200. A few minutes later the Bp. dropped to 43. Animal prostrate and in profound shock. A total of 467 cc. or 43.2 cc. per kgm. blood was taken of which 432 cc. were withdrawn during the first hour or between 11:35 a.m. and 12:25 p.m. No fluid was given at any time although the dog was very thirsty and sought eagerly for water. The dog died at 6:45 p.m. The animal would probably have recovered had he been permitted to drink water after the last bleeding at 4:20 p.m.

Protocol 4. Dog 107. Normal female. Weight 14 kgm. 2/20/33. At 2:10 p.m. pulse 90, Bp. 112., Hb. 13.9. At 2:35–2:40 p.m. withdrew 480 cc. blood, pulse 160, Bp. 98–100. At 2:45 p.m. drew 48 cc. blood. 2:50, drew 102 cc. blood or a total of 630 cc. or 45 cc. per kgm. blood withdrawn within 15 minutes. 2:55 pulse 176, Bp. 40. Animal prostrate and in shock. 3:20 p.m. pulse 160, Bp. 54. Dog slightly improved but still very weak. 4:55 p.m. pulse 188, Bp. 45. At 6 p.m. pulse 210, Bp. 48. At 6:45 p.m. Bp. 48 dog given water. She drank 250 cc. At this time the animal was in very poor condition. She could rise to her feet only with great difficulty. Blood was oozing from rectum. 7:00 p.m. or 15 minutes after giving the water, pulse 180, Bp. 67. At 7:30 p.m. pulse 180, Bp. 76. Animal very much improved. Gave 1000 cc. water in cage plus 300 grams of Ken-L-Ration. She ate the food at once. Following morning 2/21/33 at 10:15 a.m. pulse 140, Bp. 110, Hb. 9.7. Animal

seems normal in every respect. She had taken a total of 900 cc. water since 6:45 p.m. previous day. Experiment discontinued.

The remarkable recuperative power of the normal dog from massive hemorrhage is clearly shown by the data outlined in the protocols. In agreement with others, e.g., Blalock (1927), we have observed that the slower the bleeding the greater the quantity of blood which can be removed without symptoms of shock appearing. Compare protocols 2 and 4.

Two factors are chiefly responsible for the rise in blood pressure following massive hemorrhage. One is vaso-constriction, and the other is rapid transfer of fluid from the tissues and tissue spaces to the blood stream to augment the depleted plasma volume.

Protocol 4 illustrates the striking effect of fluid administration by mouth (drinking water) when the animal is in shock following hemorrhage. Note that the symptoms of shock appeared in the animal at 2:55 p.m., the Bp. at this time being 40 mm. Hg. The Bp. flucutated during the next few hours, i.e., it rose from 40 to 54 then fell to 45, and at the end of approximately four hours registered 48. During this interval no water was given, and as a result no amelioration of symptoms and no significant rise in pressure occurred. But within 15 minutes after the dog had taken 250 cc. water the pressure rose from 48 to 67 and within forty-five minutes the Bp. stood at 76—a rise of 28 mm. Hg.

It is obvious that when the hemorrhage is of great magnitude the animal must have fluids available if dilution is to occur. In such cases the blood diluting mechanism is functioning properly, but the supply of fluid is inadequate.

Hemorrhage in the adrenalectomized dog maintained on cortical hormone. Bilaterally adrenalectomized dogs were used. They had well healed wounds and were normal animals[3] at their peak weight. Anyone viewing the animals would have been unable to distinguish them, either by appearance or behavior, from control unoperated dogs. They were injected daily from the day of adrenal removal, until ready for use, with adequate doses of the cortical hormone. Some of the animals had been adrenalectomized four to six months, others for lesser periods. In all cases, however, the dogs were permitted to develop severe symptoms of adrenal insufficiency by withdrawal of extract, and then revived and returned to normal health by adequate injections of hormone. Several of the older animals used in these experiments have passed through this routine three

[3] The Hb. of bilaterally adrenalectomized dogs is generally, if not invariably considerably lower than normal. The observations relating to this anemia will form the subject of a separate communication. Suffice it to state here that our experimental data indicate that the lowered Hb. is due to adrenal removal itself and not to dietary or other disturbing factors.

or four times. When an experiment starts, the cortical hormone is withheld and the animal allowed to become prostrate from insufficiency. The hormone is then administered and the animal returned to normal health.

The shortest time interval intervening, between operation, i.e. adrenalectomy, and use of the animal for hemorrhage was approximately three weeks, the longest interval was six months.

The adrenalectomized dog such as just described, kept in normal condition by adequate doses of extract, dilutes its blood after hemorrhage, just as do normal unoperated controls. Comparatively large quantities of blood can be removed with little or no effect upon the animal. Thus, with respect to experimental hemorrhage, the adrenalectomized dog on adequate doses of hormone reacts exactly as does the control. The following protocol illustrates this point.

Protocol 5. Dog 61. Bilaterally adrenalectomized male dog. Weight 10 kgm. Had been adrenalectomized six weeks previous to use and for period of one week had been receiving 1 cc. of cortical extract per kgm. of body weight per day. Animal in perfect health on morning of the experiment. Bp. 106, pulse 88. At 10:35 a.m. drew 53 cc. of blood from femoral artery. 10:38 a.m. pulse 120, Bp. 105. At 11:00 a.m. drew 49 cc. blood making a total of 102 cc. of blood taken within 25 minutes. 11:08 pulse 146, Bp. 96. Animal in excellent condition, active and vigorous. 1:30 p.m. pulse 90, Bp. 108. The experiment was discontinued. The dog remained normal in every respect throughout the experiment and afterwards.

The chief point with regard to this protocol is that withdrawal of a total of 102 cc. of blood over a period of approximately 30 minutes had little if any effect upon the mean arterial pressure. Later this same dog off extract, and exhibiting equal vigor, activity and appetite, developed profound hemorrhagic shock when bled from the femoral artery for a total of 40 cc. and the blood pressure fell from 84 to 45 mm. Hg and did not rise again until cortical hormone was administered. See protocol 6 (fig. 1).

Hemorrhage in the adrenalectomized dog not receiving extract. The evidence seems clear that the adrenalectomized dog, kept in normal health by adequate doses of extract, dilutes his blood following hemorrhage just as the control animal does. On the other hand, if extract is withheld for a short time until the hormone content of the blood and tissues diminishes, and the dogs are then bled, they are unable to dilute. At the time of bleeding the animals appear normal in all respects except for decreased blood pressure and presumably diminished blood volume, since our data (to be published later) indicate that the pressure changes in a general way reflect volume changes in the adrenalectomized dog. The animals are active, vigorous, and eat heartily. In fact, unless the blood pressure and blood urea nitrogen, which is slightly elevated, are carefully followed, one could not distinguish the animals from unoperated controls since the

dogs are playful and seem vigorous, and the males will attempt copulation with females when the latter are in heat.

If however, as stated before, small amounts of blood are removed from such animals by way of the femoral artery, amounts which are of no significance to animals with intact adrenals, an interesting phenomenon occurs. The blood pressure rapidly sinks to a low level where it remains

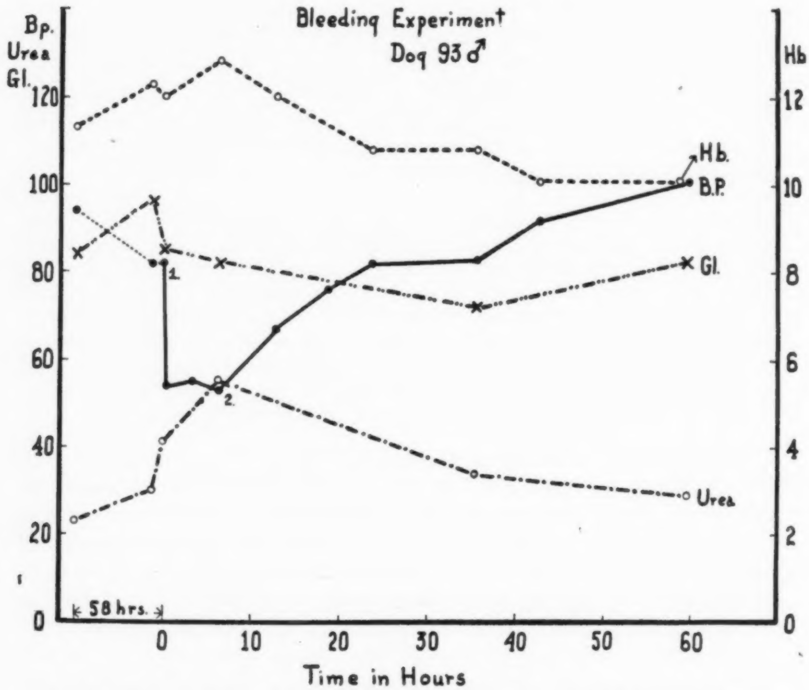


Fig. 3. The effect of slight hemorrhage in the adrenalectomized dog upon the mean arterial pressure, blood urea nitrogen, glucose and hemoglobin. The animal had been off extract for 58 hours. At *1* withdrew 4 cc. of blood per kgm., and at *2*, adrenal cortical hormone was injected. Detailed data are given in protocol 8. Bp. is in millimeters Hg; urea and glucose are given in milligrams per cent (read on left hand scale); Hb is in grams per cent (right hand scale).

unchanged. No blood dilution occurs as evidenced by failure of the arterial pressure to rise, or of the hemoglobin level to fall. Instead there is steady concentration of the blood, and unless the cortical hormone is administered the blood pressure starts to decline still further and the animals die, presenting all of the classical terminal symptoms of adrenal insufficiency and hemorrhagic shock. Following injection of adequate

amounts of hormone, blood dilution promptly occurs. The blood pressure rises to the normal level, the hemoglobin and blood urea nitrogen decline to the normal level and all symptoms disappear (see figs. 1 and 3).

The following abbreviated protocols illustrate the points mentioned. Data on several adrenalectomized animals (three dogs and four cats) studied in the early fall of 1932 are not included here, since at that time we were interested only in the Bp. following slight hemorrhage.

Table 1 is a summary of the hemorrhage experiments in the adrenalectomized dogs. The animals were all thoroughly trained for blood pressure work before use in these experiments. The last four dogs listed in the table were cases which terminated fatally. Two dogs, 68 and 91, were

TABLE 1

Summary of hemorrhage experiments in adrenalectomized dogs

DOG NUMBER	WEIGHT	NORMAL BLOOD PRESSURE	DAYS OFF EXTRACT	BLOOD PRESSURE AT TIME OF HEMORRHAGE	HEMORRHAGE	DURATION OF HEMORRHAGE	BLOOD PRESSURE AT END OF HEMORRHAGE	HOURS BEFORE EXTRACT	BLOOD PRESSURE AT TIME OF INJECTION	BLOOD PRESSURE 48 HOURS LATER
	kgm.				*cc./kgm.*					
61	10	102	3+	84	4.0	10 minutes	45	6	46	100
65	10	110	4−	78	6.3	80 minutes	47	3+	43	106
75	12.3	108	10	74	4.4	35 minutes	52	7−	53	100+
93	11.9	94	2	82	4.03	5 minutes	54	6	53	101
94	10.0	93	2	83	6.0	15 minutes	56	7	54	98
120	8.2	113	5+	75	7.2	20 minutes	51	9	53	100
122	8.6	112	3+	81	4.6	20 minutes	45	9	53	103
68	11.9	112	5−	74	7.7	13 minutes	38			
84	11.8	109	6+	89	6.4 + 4.2	48 hours	46	6		
91	10	104	3−	92	7.0	15 minutes	78			
104	9.5	97	3	80	5.26	15 minutes	52	2	46	

intentionally permitted to die. Dog 104 was moribund at the time of injection with extract. Dog 84 was used for hemorrhage before the Bp. had declined to the critical level 86 to 80 mm. Hg.

Protocol 6. Dog 61. Male: Bilaterally adrenalectomized 11/2/32. Maintained on adequate dose of cortical hormone until 11/26/32, 1 cc. per kgm. per day. On this date the dog was normal, pulse 82, Bp. 102, Urea 25. Last injection hormone this date. 11/29/32 pulse 134, Bp. 93, Urea 32. Dog very active and vigorous and with undiminished appetite. Weight 9.9 kgm. 11/30/32 pulse 145, Bp. 84, Urea 36. Animal appeared perfectly normal; ate full ration. 10:20 a.m. removed 40 cc. blood by left cardiac puncture. 10:30 pulse 132, Bp. 45, animal listless and apathetic. 4:30 p.m. or 6 hours and 10 minutes later animal in shock. Pulse 122, Bp. 46. No sign of blood dilution or blood pressure rise had appeared. Urea rose from 36 just before bleeding to 54 during the six hour interval. Animal injected with cortical hormone intravenously at 4:30 p.m., given 3 cc. per kgm. since he appeared in very bad condition.

10:20 p.m. pulse 150, Bp. 60. Animal much brighter and more active. 12/1/32 at 9 a.m. pulse 152, Bp. 79–80. Urea 45. Dog ate normal ration. Again injected with extract 12/2/32 at 9:30 a.m. pulse 108. Bp. 99, Urea 37. Animal seemed perfectly normal, active and vigorous. On 12/3/32 at 10:20 a.m. pulse 94, Bp. 100. Urea 30 at 11:30 p.m. pulse 90, Bp. 106. Urea 23. Experiment discontinued.

Protocol 7. Dog 94. Male. Weight 10 kgm. Bilaterally adrenalectomized 1/25/33. Given adequate maintenance dose cortical hormone, 1 cc. per kgm. per day until 2/4/33. Animal in perfectly normal condition on this date; eats heartily, is active and vigorous. Pulse 72, Bp. 93, urea 19, Hb. 11.5, glucose 79. Last injection on this date. 2/6/33 pulse 124, Bp. 83, urea 22, Hb. 12.4, glucose 83. At 2:20–2:35 drew 38 cc. blood. Bp. 72, pulse 130 at 3:00 p.m. drew additional 22 cc. blood or a total of 60 cc. taken. 3:10 p.m. pulse 132, Bp. 56, Hb. 12.7. Animal shows signs of shock and prostration. 8:10 p.m. or 5 hours later, animal very weak and still in shock. No signs of improvement. Pulse 132, Bp. 55. Since the dog appeared to be failing rapidly and there was no sign of blood dilution or change in blood pressure, extract was deemed necessary. At 9:40 p.m. just before extract injected, pulse 132, Bp. 54, urea 33, Hb. 12.8, glucose 89. Clinically the animal was prostrate. Intravenous injection cortical hormone given 9:45 p.m. or 6 hours and 40 minutes after blood was taken, 3 cc. extract per kgm. body weight. 2/7/33 at 9:00 a.m. the dog was up and about, ate food eagerly and had taken 300 cc. water during the night. Pulse 104, Bp. 79, urea 32, Hb. 11.4, glucose 90. Injected again with cortical hormone, intraperitoneally at 9:40 a.m. At 8:50 p.m. pulse 100, Bp. 86, Hb. 10. Animal appeared normal in every way. Again injected intraperitoneally with cortical hormone. Drank 120 cc. water during the day. 2/8/33 at 9:30 a.m. dog excellent condition. Lively and whining for food. Had taken 140 cc. water during the night. Pulse 112, Bp. 98, urea 26, Hb. 10.5, glucose 83. Injected as usual. 2/9/33. Dog normal. Pulse 96, Bp. 93, urea 22, Hb. 10.6, glucose 72. Experiment discontinued.

Protocol 8. Dog 93. Male. Weight 11.9 kgm. Bilaterally adrenalectomized 1/28/33. Maintained in normal health by adequate doses of cortical hormone until 2/4/33. Animal perfectly normal, is active, vigorous and eats full ration. Last injection hormone given this date. Pulse 80, Bp. 94, urea 23, Hb. 11.2, glucose 84. 2/6/33 at 9:15 p.m. pulse 120, Bp. 82, urea 41, Hb. 12, glucose 85. Animal active and vigorous. Withdrew 48 cc. blood by cardiac puncture. Pulse 116, Bp. 54. Animal in deep shock. Weak and spastic. At 12:15 a.m. pulse 124, Bp. 54. At 3:10 a.m. pulse 128, Bp. 53. During the interval between 9:15 p.m. and 3:10 a.m. the dog had taken 50 cc. water. At 3:30 a.m. animal in such serious condition that extract was injected intravenously, 3 cc. per kgm. Just previous to injection the pulse was 128, Bp. 53, urea 55, Hb. 12.8, glucose 82. A few hours after injection the dog was much brighter and became active. Ate 400 grams of Ken-L Ration and drank 220 cc. water. At 9:50 a.m. of 2/7/33. Pulse 130, Bp. 67. All shock symptoms had disappeared. Injected with 18 cc. extract 4:00 p.m. At 9:00 p.m. pulse 120, Bp. 82. Animal looks and acts like normal dog. 2/8/33 at 9:40 a.m. Pulse 128, Bp. 82–84, urea 34, Hb. 10. Dog had developed diarrhea during night. Lost fluid and had not taken any water. 4:30 p.m. pulse 120, Bp. 92, Hb. 10. Following day 2/9/33 at 9:45 a.m. pulse 120, Bp. 101, urea 29, Hb. 10, glucose 83. Dog normal. Experiment discontinued.

Protocol 9. Dog 75. Male. Weight 12.3 kgm. Bilaterally adrenalectomized 1/1/33. Animal maintained in normal health by adequate daily injections of cortical hormone. 1/27/33 pulse 104, Bp. 108, urea 20, Hb. 10.8, glucose 84. Last injection this date. 2/6/33 pulse 148, Bp. 74 urea, 41, Hb. 13.8, glucose 80. Animal off extract for 10 days but had shown gradually decreasing Bp. On this date dog was active,

vigorous, ate full ration. At 9:20–9:55 a.m. withdrew total 55 cc. blood. Bp. fell to 52 within five minutes. Dog exhibited symptoms of shock and prostration, was very weak and spastic. At 4:30 p.m. pulse 86, Bp. 52, urea 52, Hb. 13.9, glucose 83. No sign of blood dilution or improvement in symptoms during the 7 hour interval. Dog had taken 160 cc. water. The clinical condition of the dog was such that extract (3 cc. per kgm.) was injected at 4:55 p.m. Dog in utter collapse and unable to rise to feet. 10:30 p.m. Dog up and about eating meat and drinking water. Pulse 140, Bp. 71. 2/7/33 at 9:00 a.m. animal bright and vigorous. No symptoms. Pulse 140, Bp. 84, urea 35, Hb. 10.0, glucose 77. Animal had taken 500 cc. water during the night and had eaten food. 4:05 p.m. pulse 132, Bp. 94. Dog normal. Injected with 17 cc. extract intraperitoneally. 8:45 p.m. pulse 130, Bp. 98, Hb. 9.4. Dog took additional 370 cc. water. 2/8/33 at 9:15 a.m. pulse 104, Bp. 100, urea 23, Hb. 9.1, glucose 87. Experiment discontinued. Dog appears and acts perfectly normal.

Protocol 10. Dog 122. Female. Weight 8.6 kgm. Bilaterally adrenalectomized 4/18/33. Kept in normal health by daily injections of cortical hormone. 5/2/33 at 9:45 a.m. pulse 98, Bp. 112, Hb. 11.9, urea 19, glucose 90. Last hormone injection on this date. 5/5/33 at 9:55 a.m. animal bright, active and vigorous, appears and acts normal. Pulse 116, Bp. 81, Hb. 14.3, urea 40, glucose 83. Between 10:30 and 10:50 a.m. removed 38 cc. blood. Bp. dropped from 81 to 45 within five minutes, pulse slowed to 78. Animal in shock and prostration. Taken off table and placed in cage. At 11:30 a.m. Bp. 51, pulse 88. At 7:30 p.m. or approximately 9 hours after bleeding animal still in shock. Symptoms unchanged. Pulse 104, Bp. 51–53, Hb. 14.4, urea 47, glucose 71. Dog had taken 100 cc. water during the 9 hour interval since bleeding. 8:00 p.m. injected 3 cc. per kgm. of extract intravenously. 5/6/33 at 9:20 a.m. pulse 128, Bp. 85. Animal appears strong and active. Had taken 70 cc. water since previous evening. Injected with 12 cc. extract intraperitoneally 8:00 p.m. pulse 140, Bp. 95. Animal normal in every respect so far as vigor, activity and appetite concerned. Again injected 12 cc. extract intraperitoneally. 5/7/33 at 9:30 a.m. pulse 132, Bp. 103, Hb. 13.0, urea 33, glucose 88. Had taken 400 cc. water since 10:00 a.m. of 5/6/33. Injected with same kgm. dose of extract. 5/8/33 at at 9:35 a.m. pulse 140, Bp. 106, urea not determined. Experiment discontinued. Dog normal.

Protocol 11. Dog 104. Male. Weight 9.5 kgm. Bilaterally adrenalectomized 3/20/33. Maintained in normal health by injections of hormone. 6/9/33 extract discontinued. Pulse 100, Bp. 97. 6/10/33 pulse 136, Bp. 92. On 6/12/33 at 10:45 a.m. pulse 140, Bp. 80. Dog appears normal, eats, and is active and vigorous. 11:20 removed 50 cc. blood from the jugular vein over a period of 15 minutes. At 11:35 a.m. pulse 144, Bp. 52. Animal in shock, can barely stand on feet. 1:20 p.m. dog in profound shock, can not rise to feet. Pulse 160, Bp. 46. Injected intravenously with extract but the dog died within an hour. This animal was one of two (the other not recorded here but results were similar) which were bled from the venous side of the circulation.

Discussion. The data reveal in a striking manner the prompt reaction of the adrenalectomized dog in shock and collapse from hemorrhage, to administration of the cortical hormone. The rise in mean arterial pressure, fall in hemoglobin and blood urea nitrogen are invariable responses to extract injection. In several experiments, not recorded here, injection of normal saline solution (3 cc. per kgm.) equal in amount to the cortical hormone given, were without effect either upon the symptoms or the blood

constituents. In the absence of the cortical hormone fluid taken by the adrenalectomized dog during the period of profound shock is without significant effect upon arterial pressure or hemoconcentration. Protocols 9 and 10. The doses of cortical hormone given were in most cases excessive, and very probably beyond the requirements of the animal, but it must be remembered that the animals were in critical condition, and we did not care to run the risk of sacrifice of the dogs for the sake of determining the minimum dose needed to revive them from hemorrhagic shock.

It will be noted, however, that in those cases where the determinations were made, the rise in blood pressure was accompanied by a fall in Hb., and blood urea nitrogen concentration. The relation between Bp. and Hb. is significant, indicating that in the syndrome of adrenal shock in the dog, the blood pressure can, in a general way, be utilized as a rough gauge of blood dilution, taking place in the animal receiving cortical hormone. It is of course recognized that blood pressure is maintained by a combination of factors such as ventricular contraction, peripheral resistance, blood volume, viscosity and vasomotor changes. A radical change in any one of these various factors may profoundly alter the level of blood pressure. However, in adrenal insufficiency the blood pressure changes can be employed as a crude gauge of blood and plasma volume changes, since in the adrenalectomized animal, the blood pressure changes are primarily due to, and dependent upon volume changes. The effect of fluid administration to the animal in profound hemorrhagic shock and receiving extract treatment is further evidence of such dependence (see protocols 2 and 4).

The probable explanation for the striking effect which follows the withdrawal of small amounts of blood from the adrenalectomized dog, off extract, but to all appearances normal and vigorous, is to be found in Porter's (1925) and others' conception of a critical level of blood pressure. In the animals off extract, the blood and plasma volume are greatly lowered, and all of the compensatory mechanisms for maintaining the blood pressure, such as vasoconstriction, increased heart rate, etc., are working at their maximum efficiency to offset the diminished volume of fluid in circulation. When the blood pressure of the adrenalectomized dog is between 86 and 80 mm. Hg according to our data, the critical level has been reached, and as Porter (1925) states, "the arteries are partly empty and to empty them still further may be mortal." After the critical level of blood pressure has been reached, the loss of small amounts of blood may drop the mean arterial pressure as much as 1 mm. Hg for each cubic centimeter taken from the femoral artery. Blood volume studies, made by us using the modified Welcker method indicate that when the blood pressure of the adrenalectomized dog stands at 80 mm. Hg, the total calculated blood volume has been reduced 40 to 45 per cent. These data will form the

subject matter of another communication (Swingle, Pfiffner, Vars, Bott and Parkins, 1933).

It is evident that the clinical picture occurring in the adrenalectomized dog after bleeding is identical in all respects with that of hemorrhagic shock in the normal dog. The cause of the shock symptoms is the same in both types of animal, namely, depletion of the blood and plasma volume to the point where the quantity of fluid in circulation is inadequate.

We have repeatedly attempted in this laboratory to bleed normal unanesthetized dogs to the point where they can no longer dilute or compensate, and to keep them in profound hemorrhagic shock for some hours as we are able to do with the adrenalectomized dog. We had hoped thereby to be able to test the effect of the cortical hormone on hemorrhagic shock in normal dogs. Unfortunately, however, we have not been able to do this. In the normal unanesthetized dog the points where dilution ceases and death occurs are so close that we have not been able to separate them. The animal either dilutes or else dies promptly from the excessive blood loss. In other words, the normal unanesthetized dog with adrenal glands intact, must be bled to the death point before his diluting mechanism fails to compensate.

We are confronted with the fact that in the adrenalectomized dog off extract, although conditions are apparently extremely favorable for blood dilution and passage of fluid from tissues to blood stream, yet no such transfer takes place and dilution does not occur. The conditions in the adrenalectomized dog favoring blood dilution are low arterial pressure, low capillary pressure, slow blood flow, and relative increase in plasma colloids. Despite these favorable conditions dilution occurs only in the presence of the adrenal cortical hormone. The evidence for this statement is abundant in each protocol.

The outstanding feature of the adrenalectomized animal is the loss of its ability to maintain a normal volume of circulating fluid in the blood stream. These animals are continually losing fluid (and salt Loeb, 1933; Harrop et al., 1933) from the blood. Considerable fluid is lost from the body as a whole by way of the urine, feces and lungs. The important point, however, is not the fact that the adrenalectomized dog continually loses fluid from the blood stream—every normal dog does also—but that the animal is unable to return fluid to the blood and hold it in circulation in the absence of the cortical hormone.

The writers do not believe, nor have they ever implied in any publication, that the permeability of the capillaries is significantly changed from the normal in the adrenalectomized dog. The cortical hormone per se is probably not directly concerned with capillary permeability but with mobilization of fluid and salts for exchange to the blood stream.

Recent preliminary experiments performed in this laboratory reveal that the colloid osmotic pressure of the blood of dogs dying of adrenal insufficiency is within the normal range. This is more or less what one would expect considering the hemoconcentration present in such animals and the increased plasma protein concentration. The fact that blood dilution does not occur in the adrenalectomized dog off extract, despite the extremely favorable conditions for its occurrence, and that dilution promptly occurs following adequate injections of cortical hormone, indicates, to us at any rate, that the adrenal cortical hormone is a necessary part of the mechanism of dilution.

We have abundant evidence (data as yet unpublished) that the adrenalectomized dog, prostrate from adrenal insufficiency and on the point of death, with greatly diminished volume of circulating blood, extremely low arterial pressure, and marked hemoconcentration, does have sufficient salt and water in the tissues and tissue spaces to bring about revival to the point where the animal appears and acts like a normal dog in so far as appetite and activity are concerned. Despite the total lack of fluid and food intake, such animals when injected with cortical hormone rapidly dilute their blood, and the Bp. may rise 20 to 30 mm. Hg within a few hours, the Hb. falls and the clinical symptoms disappear. The Bp. and Hb., however, do not return to the normal level.

We have mentioned *1*, that the colloid osmotic pressure of the blood of the adrenalectomized dog remains normal, even when the animal is practically moribund; *2*, that such animals have adequate salt reserves to completely restore them to normal; and *3*, sufficient fluid to revive them to a condition approximating normal. The only explanation which appears to satisfactorily account for the facts (data pertaining to points 1, 2, 3, just stated are now in preparation for publication) is that in the absence of the cortical hormone, the water and salt content of the blood progressively diminishes by transudation and is no longer freely mobile. Considerable fluid may be lost in the urine since the urine output of the adrenalectomized dog (extract withheld) is maintained within the normal range during the fore period before active symptoms appear, and drops sharply with the appearance of symptoms. We have been unable to confirm Loeb et al. (1933) observations that the urine volume is greatly augmented during the fore period before the onset of symptoms.

The fluid and salt (Loeb et al., 1933; Harrop et al., 1933) drained out of the blood are as definitely lost from circulation as those passed out in the urine and other excretions, since they are no longer available and can not be mobilized in the absence of the cortical hormone. It appears then, that the chief rôle played by the hormone in the mechanism of blood dilution, is that of a mobilizer of salt and water in the tissues and interstitial spaces for transfer to the blood stream. Just how the cortical hormone mobilizes

the water and salt in the adrenalectomized dog so that fluid transfer from tissues to blood, i.e., dilution, can take place, is not clear.

It should be pointed out that in these experiments no significant alterations of the blood glucose have been observed. This fact, quite clearly brought out in the protocols (fig. 3), renders invalid any assumption that the experimental shock induced in the adrenalectomized dog by slight hemorrhage is a result of hypoglycemia.

SUMMARY AND CONCLUSION

1. Normal unanesthetized dogs, bled to the extent of 40 to 54 cc. per kilogram of body weight and thereby thrown into profound shock, rapidly dilute their blood and return to normal condition within a relatively short time.

2. Complete restoration to normal condition, e.g., blood pressure and hemoglobin, etc., of animals bled to this extent necessitates a fluid intake (by mouth) of several hundred cubic centimeters of water. The fluid reservoirs of the tissues and interstitial spaces are inadequate to make good the deficit caused by such massive hemorrhage.

3. The bilaterally adrenalectomized dog kept in normal health by adequate doses of cortical hormone, reacts to hemorrhage by rapid dilution of the blood, just as does the normal unoperated animal.

4. The adrenalectomized dog off extract but still in the period of good health and free from symptoms of any kind reacts to trivial blood loss by developing profound hemorrhagic shock. However, the normal unanesthetized animal with adrenal glands intact, must be bled to the death point before the blood diluting mechanism fails to compensate.

5. The injection of adequate amounts of cortical hormone into adrenalectomized animals in profound hemorrhagic shock results in blood dilution. The blood pressure rises to the normal level, the hemoglobin and blood urea nitrogen decline, and all symptoms of shock disappear.

6. The clinical signs and symptoms induced in the adrenalectomized dog off extract, by removal of small quantities of blood (4 to 8 cc. per kgm. of body weight) are identical with those of hemorrhagic shock as exhibited by normal dogs after excessive bleeding.

7. The outstanding feature of the adrenalectomized dog is the loss of ability to maintain a normal volume of circulating fluid in the blood stream, and inability to dilute the blood despite the extremely favorable conditions (except lack of the adrenal cortex) for blood dilution present in such animals.

8. The rôle of the adrenal cortical hormone in the normal mechanism of dilution is that of a mobilizer of water and salt, i.e., it mobilizes the relatively protein-free saline solutions of the tissues and interstitial spaces so

that they become available for transfer to the blood stream. In the absence of the hormone such fluid transfer does not occur.

9. In the normal animal with intact, functioning adrenals, the part played by the adrenal cortex in the phenomenon of blood dilution is not apparent because the water and salt of the organism are freely mobile. In the adrenalectomized animal, however, the cortical hormone, the factor necessary for mobilization of water and base, is lacking. Without this factor, the action of the two forces, hydrostatic pressure and colloid osmotic pressure is nullified, and normal fluid exchange becomes impossible.

10. Since significant alterations in the blood glucose were not observed in this series of experiments, hypoglycemia can not be considered as playing any part in the shock syndrome studied.

REFERENCES

Adolph, E. F., M. J. Gerbasi and M. J. Lepore. 1933. This Journal, **104**, 502.
Blalock, A. 1927. Arch. Surgery, **15**, 762.
Carrier, E. B., F. W. Lee and G. H. Whipple. 1932. This Journal, **61**, 138.
Damashek, W. and J. Loman. 1932. This Journal **101**, 140.
Gesell, R. 1918. This Journal, **47**, 468.
Gradinescu, A. V. 1913. Pflüger's Arch., **152**, 187.
Harrop, G., A. Weinstein, L. J. Soffer and J. H. Trescher. 1933. Journ. Exp. Med., **58**, 1 (also 17).
Kellaway, C. H. and H. S. Cowell. 1923. Journ. Physiol., **57**, 305.
Loeb, R. F., D. Atchley, E. M. Benedict and J. Leland. 1933. Journ. Exp. Med., **57**, 775.
Lucas, G. W. H. 1925. This Journal, **77**, 114.
Meek, W. G. and J. A. E. Eyster. 1921. This Journal, **56**, 1.
Parkins, W. M. In press.
Porter, W. T. 1924–25. This Journal, **81**, 277. (Reference to author's earlier papers included.)
Rogoff, J. M. and G. N. Stewart. 1926. This Journal, **78**, 711.
Somogyi, M. S. 1926. Journ. Biol. Chem., **70**, 599.
Starling, E. H. 1896. Journ. Physiol., **19**, 312.
Swingle, W. W. 1927. This Journal, **79**, 666.
Swingle, W. W., J. J. Pfiffner, H. M. Vars, P. A. Bott and W. M. Parkins. 1933. Science, **77**, 58.
Viale, G. and A. Bruno. 1927. Comptes Rendus, Soc. de Biol., **97**, 261.

AN ATTEMPT TO USE CHRONAXIE AS A MEASURE OF EXCITABILITY

JOHN W. BEAN

From the Department of Physiology, University of Michigan, Ann Arbor

Received for publication October 2, 1933

In view of the more or less general acceptance of chronaxie as a measure of excitability it was thought that it might be used to advantage in the study of certain problems concerning respiration and its control. The work summarized in this report was begun then, not essentially as a study of chronaxie itself, but rather as an attempt to test the feasibility of its application to the problems in question.

The chronaximeter used in these experiments has been described (Gesell, 1933). The cathode was an Ag-AGCl-Ringer's solution electrode of the capillary type with a bore of 0.4 mm. diameter which brought it within the range specified by Lapicque (1932).

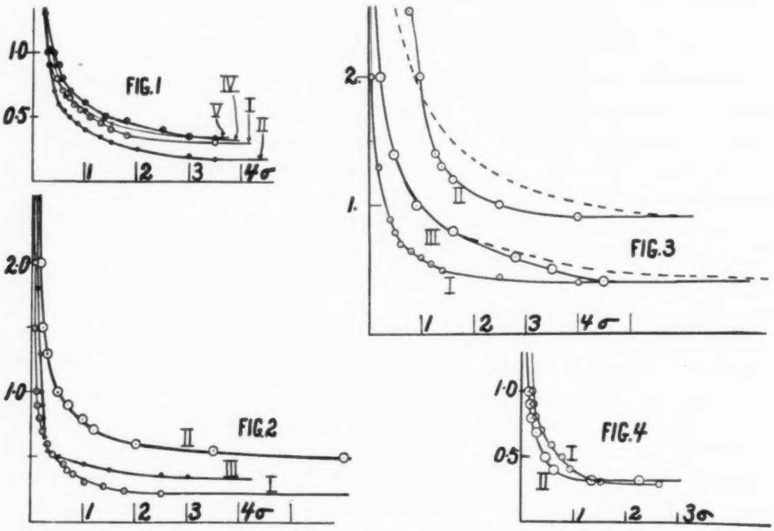
The dogs used in these experiments were anesthetized with morphine and urethane. The sartorius muscle was exposed over its distal end and a small clear glass funnel was inverted over the exposed muscle and its edge inserted below the skin. The capillary cathode carried on and adjusted by a manipulator was applied to the muscle at right angles (Lapicque, 1932) through the small end of the funnel which was loosely packed with moistened cotton. The indifferent anode was applied to the muscle a short distance from the cathode but never less than 1 cm. as recommended by Lapicque (1932)—in the later experiments it was placed on the chest. Locating the cathode on the distal end of the muscle gave reasonable assurance that motor nerve fibers would not be involved in the stimulation.

In order to ascertain whether a determined chronaxie is the true chronaxie the strength duration curve must be available for comparison with the "Canonic" curve (Lapicque, 1931). In the early experiments of the present investigation data for plotting the full strength duration curves were obtained. It developed, however, that this method of determining chronaxie involved certain inherent disadvantages so in later experiments the more usual method, viz., that of determining chronaxie at a single point, was resorted to.

RESULTS. In figure 1 are shown four strength duration curves representing successive series of readings. Each series covered a period of about twenty-five minutes. The rheobase and chronaxie values as determined

from the curves (fig. 1) are given in table 1. Curve III was omitted from the figure for simplicity.

On examination of these curves it was found that the second normal curve (II) did not conform with the canonic curve. If the second normal curve (II) be eliminated on these grounds the initial decrease of chronaxie with hemorrhage becomes practically negligible and the second post hemorrhage chronaxie (1.00) was increased over the initial normal (0.75). Reinjection of the defibrinated withdrawn blood brought about a decrease in chronaxie but a slight increase in rheobase.



Figs. 1 to 4

The cause for the non-conformance of one of these curves with the canon cannot be reasonably attributed to physical factors—which according to Lapicque may give rise to pseudo chronaxie. It would seem that the non-conformance is to be explained on the basis of a changed physiological state of the tissue. If this be so, we would be led to acknowledge that there must exist physiological states for which true chronaxie values cannot be determined. Moreover such states may be arrived at quite unknowingly.

The results of an experiment on another animal are summarized in table 2. (Fig. 2.)

Examination of the curves from which these values were obtained revealed that none of the three was strictly canonic but the post hemorrhage curve approached the canonic form very closely. Apparently hemorrhage

transformed a non-canonic to an approximation of the canonic curve and reinjection brought about a return to the non-canonic form. If we are to use only true chronaxies as defined by Lapicque we are left no valid data from this experiment.

Turning to a consideration of the rheobase values as given in table 2 it is seen that in none of these curves was the rheobase at the end of each series the same as it was at the beginning of that series. This inconstancy of the rheobase obviously brings the validity of the chronaxie recorded from such curves into question. It might be expected that the curve in which the initial and final rheobase values differed most widely would

TABLE 1

	RHEOBASE	CHRONAXIE	CURVE
	volts	*sigma*	
Normal 1	0.29	0.75	I
Normal 2	0.18	1.30	II
Hemorrhage, 150 cc. (1.9 per cent of body weight)			
Post hemorrhage 1	0.30	0.60	III
Post hemorrhage 2	0.30	1.00	IV
Post reinjection, 1	0.35	0.60	V

TABLE 2

	RHEOBASE, BEGINNING OF SERIES	CHRONAXIE	RHEOBASE, END OF SERIES
	volts	*sigma*	*volts*
Normal	0.23	0.60	0.25
Hemorrhage, 200 cc. (2.5 per cent of body weight)			
Post hemorrhage	0.50	0.50	0.40
Post reinjection	0.35	0.25	0.32

diverge most markedly from the canonic form. Contrary to this expectation it was found that this curve approximated the canonic form most closely. A progressively changing rheobase is then not necessarily a contributing factor to the non-canonic form of curve and even though a strength duration curve does conform with the canon the validity of the chronaxie may still be questioned on the basis of an inconstant rheobase.

The changes in rheobase as a result of hemorrhage and reinjection are worthy of note. It is seen from table 2 that rheobase as recorded at the beginning of each series was increased by hemorrhage and decreased with reinjection. A similar reversible change is recorded for the final rheobase readings of each series. This suggests that perhaps rheobase itself is just as reliable if not a more reliable index of excitability than chronaxie.

In figure 3 (table 3) are shown three curves from another experiment. The broken lines indicate the canonic curves as they would lie for the same chronaxie and rheobase values. In this experiment the chronaxie was determined not only from strength duration curves but also by the single point method at the beginning and end of each series of strength duration readings.

A study of the chronaxie values as determined from the curves and from single points at the beginning and end of each curve shows that hemorrhage increased the chronaxie as read from the curve and from single points and

TABLE 3

	RHEOBASE, BEGINNING	CHRONAXIE, FROM CURVE	CHRONAXIE		RHEOBASE, END	CURVE
			Beginning	End		
	volts	*sigma*	*sigma*	*sigma*	*volts*	
Normal	0.40	0.50	0.50	0.80	0.78	I
Hemorrhage 100 cc. (1.0 per cent of body weight)						
Post hemorrhage	0.90	1.10	1.20	1.50	1.10	II
Reinject 100 cc. defibrinated blood + 100 cc. isotonic saline solution						
Post reinjection	0.40	1.60	0.90	1.00	1.05	III

TABLE 4

	RHEOBASE, BEGINNING	CHRONAXIE, FROM CURVE	RHEOBASE, ENDING
	volts	*sigma*	*volts*
Normal:			
1st curve	0.30	0.70	0.35
2nd curve	0.30	0.75	0.35
Post hemorrhage	3.00	1.30	3.00
Post reinjection	0.80	0.10	

that reinjection brought about a reversal of the hemorrhage effect so far as the single point values were concerned but a further increase in chronaxie as determined from the strength duration curve.

Which of these values—if any—should be accepted as indicating the state of excitability of the muscle? Application of the canonic test proved the post hemorrhage and post reinjection curves to be non-canonic. And although the single point values of chronaxie here recorded are not subject to the error which might be introduced by an inconstant rheobase, we have no way of determining whether they are true or pseudo chronaxies.

More striking changes in rheobase as a result of hemorrhage and subse-

quent reinjection are shown in table 4 which summarize the results of another experiment.

The chronaxie values were determined from strength duration curves which did not comply with the canonic requirements and so are not true chronaxies although they do illustrate a reversible change.

The fact that carbon dioxide is an important metabolite not only of muscle but tissues generally would seem to justify the supposition that muscular excitability would be affected by its administration to the animal in strong gaseous mixtures. Gay (1931) found that carbon dioxide administered in gaseous mixtures of from 5 per cent to 25 per cent in pure oxygen decreased the response of the sartorius muscle to submaximal stimulation from which he concluded that irritability of muscle was decreased by an increase of acidity.

With these results in mind experiments were carried out in which the animals were made to breathe gas mixtures high in carbon dioxide content. Figure 4 (table 5) shows two curves: I, the normal taken during room air administration, and II, drawn from a series of readings made while the animal was breathing a 10 per cent mixture of CO_2.

TABLE 5

	RHEOBASE, BEGINNING	CHRONAXIE	RHEOBASE, END	CURVE
	volts	*sigma*	*volts*	
Normal (room air)	0.32	0.50	0.32	I
CO_2 (10 per cent)	0.33	0.35	?	II

Before the end of the second series of readings (i.e., the CO_2 series) had been completed the animal died from too long an administration of the CO_2 mixture. The last two readings for the curve were made after cessation of respiration, heart beat and with blood pressure at zero. One might expect there would have been a pronounced change in excitability with such an extreme alteration in the condition of the animal but the change in chronaxie—a decrease—was surprisingly small. The normal curve (I) proved to be of the canonic form and the rheobase remained constant throughout the series of readings but the administration of high carbon dioxide transformed the canonic normal curve to a non-canonic form (curve II).

Clearly if chronaxie is to be of value as a measure of excitability, where that excitability is progressively changing or where that change is perhaps short lived or where the rheobase is progressively changing, it must be determined by some method other than that necessitating the plotting of the strength duration curve. If we employ the single point method of making chronaxie determinations we do not know whether the derived value is a true chronaxie or not but we do eliminate the error which would be intro-

duced by a changing rheobase. It is this single point method which has been used almost exclusively in the various problems reported in the literature, and for the studies in which the use of chronaxie had been contemplated here, it seemed to be the only method applicable.

The effects of fatigue as induced by faradic stimulation of the gastrocnemius and sartorius muscles of the frog were followed. Chronaxie readings were made at intervals of about five minutes. A summary of the results of one of these experiments is given in table 6.

TABLE 6

	TIME OF DAY	DURATION OF FARADIZATION	RHEOBASE		CHRONAXIE	
			Before	After	Before	After
			volts	*volts*	*sigma*	*sigma*
1	3:55	2 seconds	0.70	0.68	0.70	0.90
2	5:05	1.25 minute	0.73	3.00	0.47	0.60
3	8:22	0.50 minute	0.70	7.50	0.37	0.80
4	10:48	0.50 minute	0.60	3.50	0.80	0.40
5	11:22	0.50 minute	0.55	2.70	0.65	0.45

TABLE 7

TIME OF DAY	RHEOBASE	CHRONAXIE	TEMPERATURE, RECTAL	TIME OF DAY	RHEOBASE	CHRONAXIE	TEMPERATURE
	volts	*sigma*			*volts*	*sigma*	
5:09	0.60	0.55	37.5	5:50	0.75	0.50	36.9
5:13	0.60	0.50		5:55	0.75	0.45	
5:17	0.60	0.55		6:01	0.95	0.30	36.6
5:20	Hemorrhage			6:09	1.00	0.22	36.4
5:22	0.65	0.50	37.5	6:14	1.90	0.25	36.4
5:25	0.60	0.45		6:20	1.75	0.27	
5:29	0.70	0.50		6:26	1.77	0.15	36.3
5:30	Hemorrhage to death			6:33	1.80	0.25	
5:33	0.65	0.45		6:44	2.00	0.27	36.0
5:36	0.85	0.55	37.3	6:46	5.00	?	
5:38	0.85	0.52		6:50	20.00	No response	
5:45	0.85	0.50	37.0				

Such pronounced effects on rheobase were not demonstrated in all similar experiments. In some cases the rheobase decreased after faradization. This variation might be attributed in some instances to an imperceptible shifting of the cathode on the muscle during the tetanic contraction of the muscle. The consistently reversible rheobase readings given in table 6 however can hardly be explained on this basis. One of the points emphasized by those who advocate chronaxie in preference to rheobases as

a measure of excitability is that the uncertainty of rheobase readings due to an electrode shift is not met with in the chronaxie method. These experiments on fatigue do not support this belief. Moreover the results of experiments in which the cathode was raised from the tissue and again lowered to approximately its original position gave ample proof of the untenability of the belief (Fredericq, 1928) that a shift in position of the stimulating electrode does not alter the chronaxie.

It need not be argued here that the response of a tissue is altered by fatigue and there would seem to be no reason why the minimal response of that tissue and consequently the rheobase would be exempt from such fatigue effects. The rheobase changes recorded in table 6 then would not be unexpected. Lapicque and Lapicque (1919) however have found that rheobase is not altered by fatigue while chronaxie is increased, and Rushton (1933) reports that fatigue has no effect whatever on muscle threshold no matter what the nature or duration of the stimulus.

The results of an experiment—one of the final group—in which chronaxie was followed after death of the animal, are given in table 7. In this experiment death was brought about by hemorrhage in two stages. The first hemorrhage produced very little change in chronaxie and the second which brought about death had no pronounced immediate effect. One hour and ten minutes after death the chronaxie had decreased to one-half its normal value. The rapid terminal rise in rheobase made further chronaxie determinations impossible. After the last reading recorded in table 7 (rheobase of twenty volts) no response of muscle could be elicited.

The chronaxie changes with death were followed in nine dogs. In none of these experiments, with perhaps the exception of one, was there observed any increase in chronaxie. Indeed the majority of these experiments of which the one cited above is typical, gave results which indicated an increased excitability for periods as long as an hour after death and this, in the face of decreasing rectal temperature.

In some of these experiments it was not unusual to find an initial decrease in rheobase just preceding and in the very early stages of death and it is not inconceivable that there does occur some exaltation of excitability. In this connection it is interesting to note that Gay (1930) found impaired oxidations brought about by administrations of sodium cyanide and gaseous mixtures low in oxygen gave rise to an increase in response of the sartorius muscle in the dog. Moreover a study of some of Mr. Gay's unpublished records revealed that the response of the sartorius muscle of the dog was often increased immediately after death and remained above or equal to the normal response for some time, then decreased. These changes in response after death would (accepting response as an index of excitability) point to an early increase and a later decrease in excitability of the sartorius muscle. Again if we assume that an increase in rheobase

indicates a decrease in excitability, the values recorded in table 7, viz., an initial decrease after death then a gradual increase and finally a sharp rise, show that changes in excitability which occurred in our experiments were very similar to those which occurred in some of Mr. Gay's experiments.

The relatively small change in excitability as determined either by rheobase or chronaxie during and for some time after death may have been due to two conflicting influences, viz., impaired oxidations which increase the response of muscle and increasing acidity which decreases the response (Gay, 1930). The sharp rise in rheobase in the terminal readings suggests a rapid swing to the acid direction.

SUMMARY

The work summarized in this report was begun, not as a study of chronaxie itself but rather as an attempt to test the feasibility of applying this widely accepted measure of excitability to certain problems relative to respiration and its control.

The experimental animals in most cases were dogs anesthetized with morphine and urethane. The tissue used was the sartorius muscle, the distal end of which was exposed but left in situ.

Chronaxie measurements were made both from strength duration curves and by the single point method of doubling the rheobase, during various experimental procedures.

Hemorrhage, administration of high percentages of carbon dioxide via trachea frequently transformed canonic curves into non-canonic forms and non-canonic into canonic curves. These transformations were attributed to the changed physiological states of the tissue induced by the experimental procedures. Granting this, the situation was such, that frequently the very state for which chronaxie readings were desired, precluded the possibility of obtaining true chronaxie readings.

A canonic form of curve may result even with a rheobase which changes progressively throughout a series of readings.

The argument that chronaxie is to be preferred over other methods of determining excitability because of its independence of physical factors is not supported by the results of these experiments.

The most nearly uniform chronaxie changes (single point method) were observed subsequent to the death of the animal. In these experiments the chronaxie decreased for as long as an hour and fifteen minutes after complete cessation of respiration and circulation. Rheobase frequently decreased slightly early in death then increased slowly and finally rose so rapidly as to make chronaxie readings impossible.

From the results of these experiments it was concluded that the use of

chronaxie as a measure of excitability in the problems mentioned in the introductory paragraph of this paper does not seem feasible.

REFERENCES

Fredricq, H. 1928. Physiol. Rev., **8**, 501.
Gay, H. H. 1931. This Journal, **98**, 60.
Gesell, R. 1933. Proc. Amer. Physiol. Soc., April 10, p. 36.
Lapicque, L. 1931. Journ. Physiol., **73**, 187.
1932. Journ. Physiol., **76**, 621.
Lapicque, L. and M. Lapicque. 1919. Compt. Rend. Soc. Biol., **82**, 772.
Rushton, W. A. H. 1932. Journ. Physiol., **73**, 424.
1933. Journ. Physiol., **77**, 337.

THE ASSAY OF INSULIN AND THE BLOOD SUGAR LEVEL

M. CAROLINE HRUBETZ

From The Department of Physiology, College of Physicians and Surgeons, Columbia University, New York City

Received for publication October 2, 1933

The problem of the assay of insulin has recently been reopened (1, 22, 23) and the accumulating data in our own work on the effects of insulin upon the blood sugar level of the rat confirm criticisms of the older methods. As a necessary part of our work on the assay of insulin we have attempted to establish a "norm" for the blood sugar level of the rat. In addition to the study of the blood sugar method in insulin assay, we have studied the incidence of shock after varying doses of insulin and have compared, as a measure of dosage, the relative precision of the shock method of assay with that of the measurement of the initial fall in the blood sugar after minute doses of insulin.

METHODS. All of the rats used in this research have been raised in this laboratory from the pied strain kindly supplied to us by Pennsylvania State College.[1] They were fed the normal ration for breeding (2), and kept in a room the temperature of which was maintained between 70° and 80°C.

The Somogyi modification of the Shaffer-Hartmann (3) blood sugar method for 0.2 cc. was used. The blood was obtained from the small saphenous vein.[2] All the determinations reported in this paper were made by the author.

Normal blood sugar level. To determine the "norm" for the blood sugar level, five series with a total of 481 observations were made on normal-fed rats. We have at present no explanation to offer for the variation in the means except perhaps that of a seasonal variation (4, 5, 6, 7). Because of the variations which we have found to occur in spite of carefully standardized diet and care, we conclude that a control series, made at the same time and under the same conditions, should accompany all blood sugar work on the rat. Table 1.

Age, sex and weight. Pearson's formula (8) was used for the calculation of the coefficients of correlation between the blood sugar level and age, sex and weight. From table 2 it can be concluded that the blood sugar level of the rat is independent of any of the factors mentioned.

[1] Grateful acknowledgment is made to Pennsylvania State College for the original stock which formed the basis for this colony.

[2] Method by Dr. Hannah E. Honeywell, Pennsylvania State College.

Inanition. Both the general practice of subjecting animals to a brief period of inanition preliminary to the collection of blood sugar data (7), and the necessity of depriving animals of food in the course of the present research, made it desirable to follow for several hours the effect of inanition in the rat. We have sought to determine the time of the minimum level and to establish whether the precision of the determinations could be increased in the rat as it is in the rabbit. All experiments were begun between 8:00 and 9:00 in the morning.

TABLE 1

Normal blood sugar level in the rat

SERIES	NUMBER OF OBSERVATIONS	MEAN	STANDARD DEVIATION	STANDARD DEVIATION OF MEANS
		mgm. per cent	*mgm.*	
February, 1930	99	112	10	1.0
December, January, February, 1931–32	94	99	8	0.8
	94	102	8	0.8
October–November, 1931	97	89	10	1.0
	97	91	9	0.9

TABLE 2

Correlation between blood sugar level and age, sex and weight

	NUMBER OF OBSERVATIONS	"r"
Age (2½–21 mos.)	602	0.21
Sex:		
Male	328	0.64
Female	274	0.68
Weight (100–375 gms.)	948	0.003

From table 3 one observes that inanition produces the greatest fall in the blood sugar during the first 12 hours. The level after 24 hours is nearly constant. At that time, the standard deviation is also less.

Insulin and blood sugar. It is the general opinion (5, 9, 10) that the initial lowering of blood sugar with insulin is independent of the amount of insulin given. Not much attention, therefore, has been paid to the idea of assaying insulin on the basis of the initial fall in the blood sugar level produced by any given dose of insulin. Survey of the work from this point of view reveals that not until the work of Scott and Dotti (1) has there been an adequate study of the effects of very minute doses of inuslin.

A difficulty met with in the effort to standardize insulin and one that is

continually emphasized in the literature, is the great variability in the effects produced, even when the same animal is injected with the same dose of insulin at intervals of several days. This suggests that the animal material is not standardized, though of course there is the possibility of increasing the precision by increasing the number of observations.

We have studied the effects of insulin in the rat both with respect to onset of symptoms of shock and to the initial change in the blood sugar level.

Insulin dosage. In this experiment approximately 100 observations were made on each of the following doses: $\frac{1}{32}$, $\frac{1}{16}$, $\frac{1}{8}$, $\frac{1}{4}$, $\frac{1}{2}$, 1, 2, and 4 units of insulin per kilo. Scattered throughout the series 200 control observa-

TABLE 3

Blood sugar level and inanition

	6-HOUR SERIES		REMAINING SERIES					
	Controls	Inanition	Controls	12 hours	18 hours	24 hours	36 hours	48 hours
Number of observations	97 97	96	100	100	101	105	103	102
Mean	89 92	83	112	97	98	87	93	83
Standard deviation	9.6 9.2	6.1	10	7.0	7.7	8.0	9.9	8.7
Standard deviation of mean	0.9 0.9	0.6	1.0	0.7	0.7	0.8	0.9	0.8
Relative change in per cent		7.5		13.3	12.4	20.5	16.9	25.9
Standard deviation of ratio		1.1		1.4	1.4	1.7	1.8	1.7

tions were made in which the appropriate volume of a 0.9 per cent sodium chloride solution was injected instead of the insulin. The insulin was diluted with isotonic sodium chloride solution so that 1 cc. contained the dose per kilo. The blood sample was taken $\frac{1}{2}$ hour after the animals were injected.

A total of 951 observations was made. The results, figure 1, show a mean drop of 6 mgm. $\frac{1}{2}$ hour after the injection of $\frac{1}{32}$ unit per kilo. When the changes in the sugar level are plotted as the ratios to the initial value and against the logarithm of the dose, the curve is a straight line until the $\frac{1}{2}$-unit dosage is approached. At about this region it begins to flatten out and the effect per unit upon the blood sugar of doses greater than $\frac{1}{2}$ unit becomes progressively less and less as the dose is increased, forming a

curve which is similar in contour to that observed by F. N. Allan (12) for the glucose equivalent of insulin. The Insulin Committee of Toronto observed such a curve, but owing to their great probable error, they interpreted the curve to indicate only a general trend (13). They state that in order to get dependable results from such a curve, one would have to use a very large number of observations on a very large number of animals. In this prediction they seem to have been right and apparently missed some important observations by not putting it to the test of experiment.

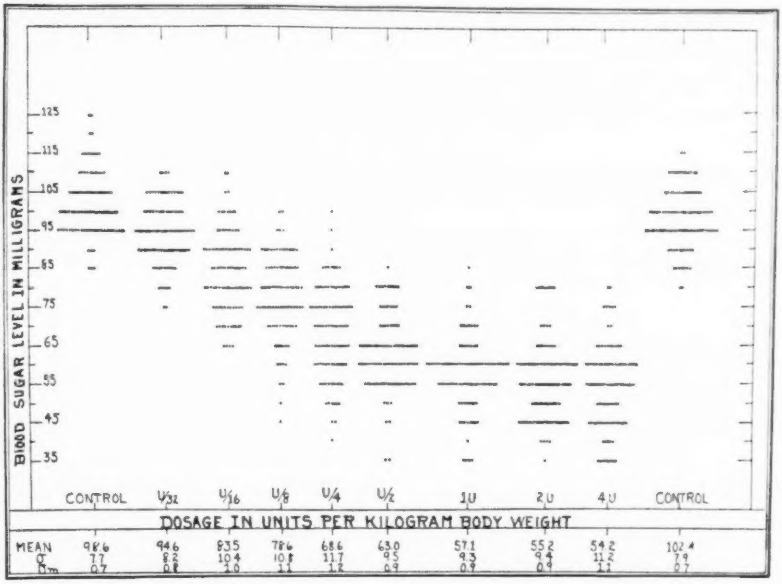


Fig. 1. Scatter chart to show the effect of increasing insulin dosage ½ hour after injection.

The flattening of the curve above the ½-unit dosage may indicate that the fermentable reducing substance is approaching zero. Somogyi (14) reports values for the non-fermentable fraction of human blood as ranging from 13 to 24 mgm. by the zinc sulfate method (15). We have found the non-fermentable fraction of rabbit blood to be around 36 mgm. and that of rat blood around 43 mgm. (unpublished). As the value for the fermentable reducing substance approaches zero, the non-fermentable reducing substance contributes a constantly increasing percentage of the total reducing power of the blood. Variations in this non-fermentable portion, then, become increasingly significant as the true carbohydrate approaches zero.

This may explain the larger and more variable deviations which were obtained with the larger doses.

Since the doses were successively doubled, the curve, as plotted, shows that the percentage drop is a logarithmic function of the dosage. Scott and Dotti (1) with 1050 observations on rabbits after doses ranging from $\frac{1}{16}$ to 2 units per kilo obtain a very similar curve. The points on their curve parallel the points shown by the series just presented up to the $\frac{1}{2}$-unit dosage, figure 2. Beyond this point, the curves begin to diverge somewhat. It will be noted that in this upper region there is a considerable loss in precision, because of the small differences of effects of considerable dose differences. The loss of parallelism at this place between our curve and that of Scott and Dotti, as well as the lack of precision encountered by

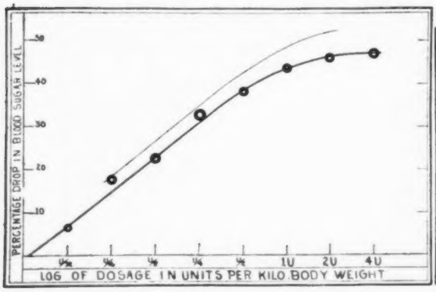


Fig. 2

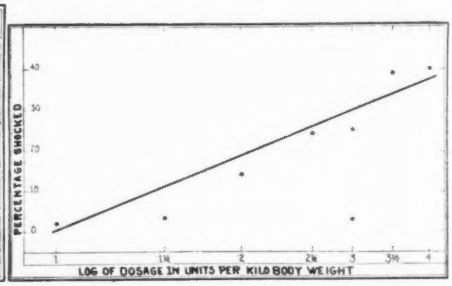


Fig. 3

Fig. 2. Graph to show the drop in the blood sugar level as a ratio of the initial value after insulin $\frac{1}{2}$ hour after injection. The heavy line represents the results of the author, obtained with rats, the fine line those of Scott and Dotti upon rabbits. The white circles represent once the standard deviation of the ratios, the black circles twice the standard deviation of the ratios.

Fig. 3. Graph to show the percentage of animals showing hypoglycemic shock after different doses of insulin.

other workers when using large doses is probably due, in part at least, to these differences.

It is an interesting fact that in all consistent attempts at assaying insulin by the initial fall of the blood sugar, the dosage has been of an amount indicated by the flattened portion of the curve. Marks (16), assaying the International Standard powder, found that with rabbits, it is essential to employ a dose smaller than $\frac{3}{4}$ unit per kilo in order to obtain hypoglycemic effects varying approximately with the dose. In order to avoid the upper region of practically unvarying effect and also to keep the dose as large as possible so that the effects should not be so small as to give undue prominence to experimental errors, he adopted a dose of $\frac{1}{2}$ unit per kilo. From our results it appears that with this comparatively small dose, the curve was beginning to flatten out, so that he was still working very near

to or, even within the region which is relatively insensitive to dose differences.

Insulin dosage and insulin shock. As the next step, the attempt was made to determine the percentage of animals showing shock symptoms with each of a series of doses of insulin. All animals were fasted 24 hours before the administration of insulin. A total of 1000 observations was made on 55 animals; 100 observations were made on each of the doses 1, $1\frac{1}{2}$, 2, $2\frac{1}{2}$, 3, $3\frac{1}{3}$ and 4 units per kilo. The animals were observed for 6 hours. In another series, 50 observations were made on each of the doses 1, $1\frac{1}{2}$, 2, $2\frac{1}{2}$ units per kilo. In this series the animals were observed for 3 hours. The criterion of shock in this experiment was convulsions, when they occurred, but when convulsions did not occur, the complete absence of response to stimulus was used. After shock and at the end of the period, $\frac{3}{4}$ cc. of a 25 per cent solution of glucose was injected subcutaneously.

Figure 3 gives the percentage of animals which showed shock plotted against the logarithm of the dose. This is in accord with Hemmingsen and Krogh (17) who found that the ideal curve relating the insulin dosage to the percentage number of convulsions is logarithmic in character. With a dose as large as 1 unit per kilo only 2 per cent showed the characteristic symptoms. Four units produced shock in 40 per cent of the cases.

A discrepancy will be noted for the 3-unit dosage, figure 3. Here, a new sample of insulin was used. One series of 100 observations had been made before we started with the new sample. In order to rule out the possibility of variations in strength of different samples of insulin, another series of 100 observations was made with the same dosage. The first sample produced shock in 26 per cent of the cases while the second sample produced shock in only 13 per cent. This would lead to the conclusion that the second sample was of less strength than the first. Upon continuing the experiment, however, with the increasing doses, $3\frac{1}{2}$ and 4 units give percentages which fall directly into the expected places obtained by extrapolation from the results produced with the first sample. In looking over the records we find that the series which gave the low percentage of convulsions caused with 3 units, was run at a room temperature of 17° to 18°C. instead of 25° to 27°C. at which the other series was made. This is in accord with Hemmingsen and Krogh (17) and Voegtlin (24) who found that if the temperature is kept constant, one obtains a smooth logarithmic curve representing the per cent of shock when plotted against the dosage.

Discussion. In accordance with the report of the International Conference on Biological Assay held at Geneva in 1926 (18) the assay of insulin is based on the amount that will decrease the blood sugar of a normal rabbit, starved 18 to 24 hours, to 45 mgm., taken arbitrarily as the convulsive level. The international unit of insulin may be defined as the quantity which produces an effect on carbohydrate metabolism equal to

that of $\frac{1}{8}$ mgm. of the standard preparation of insulin HCl. It leaves open the exact method to be used in conducting the bio-assay for, as Macleod (19) has said, "It would be hazardous at the present time, when the physiological action of insulin is so little understood, to adopt any particular method for the purpose." The usual method is to take the average of the blood sugar percentages over a period of five hours after injection and subtract this from the original blood sugar level. The results are calculated according to the formula:

$$a/b \times w/c \times 1.5 = \text{the number of units per cubic centimeter}$$

a—the difference between the normal and the average blood sugar over $1\frac{1}{2}$, 3 and 5 hours.
b—the difference between the normal and 45 mgm.
w—weight of the rabbits
c—number of cubic centimeters of insulin injected.

The use of this formula assumes that the relation of insulin to its effect is a straight line function. As far back as 1924 Macleod and Orr (20) raised objections to the use of the convulsive method for the pharmacological assay of insulin on the grounds that 1, it is only an indirect method for measuring the hypoglycemic effects, and 2, convulsions cannot be depended upon to appear at any definite blood sugar level. Clough, Allen and Root (21) report 168 blood sugar values on rabbits below 45 mgm. with only 34.5 per cent in convulsions. They conclude that the convulsive level per se is not a definite point.

In the present study, with 1000 observations on shock dosage, an attempt was made to correlate the time of incidence of shock and the dosage. The correlation coefficient was 0.06, thus giving no indication of any such relationship. This agrees with McCormick, Macleod, Noble and O'Brien (9), who report that the time of incidence of convulsions is no accurate measure of the insulin dosage.

It will be noted that with doses of 1 unit per kilo only 2 per cent of the animals showed signs of shock, with 4 units, 40 per cent. The onset of symptoms is greatly affected by the temperature. Krogh (17) defined a mouse unit as that quantity of insulin which produced collapse or convulsions in half of the injected mice stored at a constant temperature of 30°C. Accordingly, the amount of insulin necessary to produce symptoms in 50 per cent of rats, even at temperature of from 25° to 30°C., would be a tremendous dose, one far in excess of anything that would be of physiological or clinical significance. We would agree, therefore, that for rats as well as for other animals, the convulsive method is neither an accurate nor a sensitive method for determining the dosage of insulin.

In selecting a method for the determination of dosage, there are three things to be considered, namely, sensitivity, precision and accuracy. The

sensitivity of the convulsive method on rats is so low as to be useless except for massive doses. Obviously, this method is useless for measuring the effects of doses smaller than 1 unit per kilo. Even with doses as large as 4 units per kilo, less than half the animals show definite shock symptoms. The precision of this method is also small since the criterion taken from determining whether or not an animal is in shock must be purely arbitrary and difficult of definition. An animal may exhibit all degrees of shock from scarcely noticeable nervous symptoms to convulsions and complete collapse. Since it is impossible precisely to identify any stage except that of convulsions or complete collapse, only the most extreme cases can be considered. For the same reasons the accuracy of the method must be small. Though it may be possible to differentiate between doses of 1 and 2 units, or even between 3 and 4; no measurable result can be obtained within the lower dosage range, that is, with doses under 1 unit per kilo. On the other hand, by measuring the initial fall in the blood sugar with very small doses, a relatively reliable value may be obtained. If the fall is plotted as a percentage of the initial level instead of the absolute drop, the individual differences are still further smoothed out. Of course, another very important factor in obtaining a smooth curve is a sufficient number of observations to justify statistically the use of means. Accordingly, any subsequent series of observations made under the same conditions, should give a similar curve, and be parallel with it. The significance of this becomes apparent when this curve is compared with that obtained by Scott and Dotti with rabbits where every point on the curve below the $\frac{1}{2}$-unit dosage is paralleled (the fine line in fig. 2). The precision of this initial fall is further borne out by the fact that in the time curve it is also in the initial fall that the cause and effect are proportional (unpublished).

The clinical use of insulin does not involve large doses of insulin, nor does it involve the reduction of the blood sugar to the convulsive level. It aims at a precise adjustment of the blood sugar to the normal level. Since the organism is sensitive to insulin, only small amounts are necessary when compared to amounts necessary to produce shock or to lower the blood sugar to the so-called arbitrary shock level. In view of these facts, it seems to us that it is desirable to have a method of standardization in which approximately the same insulin dosage is used as will probably be used by the clinician in treating his patients. It is suggested that such a method may be found in the measurement of the initial fall in the blood sugar level after comparatively small doses of insulin, i.e., less than $\frac{1}{2}$ unit per kilogram.

CONCLUSIONS

1. Four hundred and eighty-one observations were made upon normal-fed rats in 5 series of approximately 100 each. Mean blood sugar levels of from 89 to 112 mgm. were obtained.

2. Eight hundred and three observations made during inanition show a progressive fall in the blood sugar level through the first 24 hours; after this interval the blood sugar level tends to become constant.

3. No correlation between the blood sugar level and age, sex or weight could be demonstrated.

4. Nine hundred and fifty-one observations on the fall in the blood sugar level $\frac{1}{2}$ hour after the injection of insulin in doses ranging from $\frac{1}{32}$ to 4 units per kilo are presented. With doses less than $\frac{1}{2}$ unit per kilo the percentage drop is a logarithmic function of the dosage within the range studied.

5. The results of 1000 observations on the incidence of shock after varying doses of insulin are reviewed.

6. It is suggested that the initial fall in the blood sugar level $\frac{1}{2}$ hour after the injection in doses smaller than $\frac{1}{2}$ unit per kilo be used in the assay of insulin. This supposition is made because the effects more closely simulate those sought for in the clinical use of insulin, and because of the greater reliability in measuring the results of such doses.

REFERENCES

(1) Scott, E. L. and L. B. Dotti. Arch. Int. Med., 1932, **50,** 511.

(2) Deutscher, R. A., M. Creighton and H. A. Rothrock. Journ. Biol. Chem., 1925, **66,** 401.

(3) Somogyi, M. Journ. Biol. Chem., 1926, **70,** 287.

(4) Bochkariev, P. and N. Gregariev. Biochem. Zeitschr., 1929, **215,** 495.

(5) Macleod, J. J. R. and M. D. Orr. Journ. Lab. Clin. Med., 1923–24, **9,** 591.

(6) Cori, C. and G. T. Cori. Proc. Soc. Exp. Biol. and Med., 1926, November, 123.

(7) Scott, E. L. Arch. Int. Med., 1929, **43,** 393.

(8) Chaddock, R. E. Principles and methods of statistics, p. 264.

(9) McCormick, N. A., J. J. R. Macleod, E. C. Noble and K. O. Brien. Journ. Physiol., 1923, **57,** 234.

(10) Penau, H. and H. Simmonet. Compt. rend., 1924, **178,** 2208.

(11) Grevenstuk, J. F. and E. Laquer. Ergebn. Physiol., 1925, **23,** Abteilung 2: 1–267.

(12) Allan, F. N. This Journal, 1923–24, **67,** 275.

(13) Insulin Committee of Toronto, League of Nations Rept., IV Health, **7**:CH, 398, 1926.

(14) Somogyi, M. Journ. Biol. Chem., 1930, **86,** 655.

(15) Somogyi, M. Proc. Soc. Exp. Biol. and Med., 1929, **26,** 353.

(16) Marks, H. P. League Nations Rept., IX Health, **7**:CH, 398, 1926.

(17) Hemmingsen, A. H. and A. Krogh. League Nations Rept., VII Health, **7**:CH, 398, 1926.

(18) League of Nations Rept., 111 Health, **7**:CH, 398, 1926.

(19) Macleod, J. J. R. Carbohydrate metabolism and insulin, p. 344.

(20) Macleod, J. J. R. and M. D. Orr. Journ. Pharm. Exp. Therap., 1924, **23,** 137.

(21) Clough, H. D., R. S. Allen and E. W. Root. This Journal, 1923, **66,** 461.

(22) Hemmingsen, A. M. and H. P. Marks. Quart Journ. Pharm. and Pharm., 1932, **245,** 54.

(23) Marks, H. P. Ibid., 1932, **255,** 73.

(24) Voegtlin C., et al. U. S. Pub. Health Rept., 1924, **39,** 1935.

CENTRAL EXCITATION AND INHIBITION IN REFLEX CHANGES OF HEART RATE

A. ROSENBLUETH

From the Laboratories of Physiology in the Harvard Medical School

Received for publication October 5, 1933

A quantitative estimate of central summation requires accurate determinations of both the afferent and the efferent nerve impulses. The variations of these two values may be temporal or spatial.

It is simpler to control temporal variations than to quantitate spatial changes of the input. The methods available to estimate the number of nerve fibers activated by a given submaximal stimulus are involved and only approximate. On the contrary, varying rates of maximal stimulation can be readily and accurately determined.

Only a very rough approximation may be obtained from a direct quantitation of the nerve impulses put out by a center during reflex activation. The output may, however, be indirectly determined from the responses of the effectors. Skeletal muscle is not a suitable indicator, for it presents relations between spatial and temporal summation which are unsatisfactory for the purpose—i.e., the same tension may develop when different numbers of nerve impulses are delivered through variable numbers of nerve fibers discharging at different frequencies (Rosenblueth and Rioch, 1933). Autonomic systems, on the other hand, are satisfactory, since it has been shown that the responses are here a known function of the number of nerve impulses delivered per unit time, regardless of the number of nerve fibers involved (Bishop and Heinbecker, 1932; Rosenblueth and Rioch, *loc. cit.*).

For these reasons, in the present quantitative study of central summation the reflexes chosen were the cardiac reflexes.

Method. Cats were used, under dial anesthesia. The heart rates were recorded on a kymograph by means of a Marey tambour applied to the intact thoracic wall. The nerves were stimulated maximally through shielded buried electrodes by brief (1 σ) rectangular shocks from an improved "multivibrator" (see Rosenblueth, 1932). The rate of stimulation was recorded.

The afferent nerves used were the left vagus, the depressor and the sciatic. The effects of their stimulation were studied in preparations with both the right vagus and the accelerators intact, and in hearts connected

exclusively with either the vagus or the accelerators. The vagi were approached in the neck, the stellate ganglia through the right second or third intercostal space.

RESULTS. A. *The time-course of direct and reflex responses.* Unlike muscular contractions, which plot directly against time on the kymograph or film, changes in heart rate must be derived from the records to obtain a satisfactory graphical representation of their time-course. A quite accurate curve can be drawn by plotting the reciprocal of the intervals between two or more successive beats against the time, corresponding to the middle of those intervals (see Rosenblueth, 1932, for an application of this method to the responses of the submaxillary gland). Figure 1 illustrates the

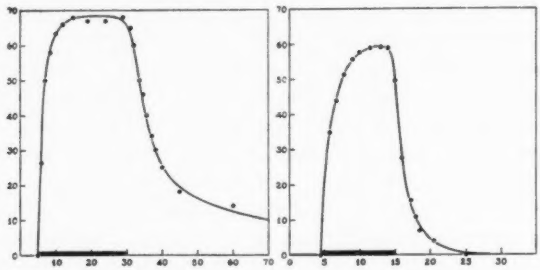


Fig. 1a Fig. 1b

Fig. 1A. Reflex slowing of the heart on central stimulation of the left vagus and depressor; right vagus and accelerators intact. Frequency of stimulation: 20 per second. Ordinates: per cent slowing calculated by taking the reciprocals of the distances in the record between 3 consecutive beats. Abscissae: time in seconds. The stimulus is marked at the bottom in this and other figures.

B. Slowing of the heart on direct stimulation of the right vagus; vagi cut, accelerators intact. Frequency: 5 per second. Ordinates: per cent slowing as in A. Abscissae: time in seconds.

curves derived by this method; A, the reflex slowing obtained by central maximal stimulation of the left vagus and depressor nerves, at the rate of 20 shocks per second; and B, the response to direct peripheral stimulation of the right vagus at a frequency of 5 per second.

An alternative simpler procedure consists in plotting the number of beats occurring during a given interval, e.g., 5 seconds, against the corresponding time. This approximation is satisfactory when dealing with slow changes, such as the return to the basal rate after a reflex response (see section C). Figure 6 illustrates curves thus constructed.

The reflex responses, both acceleration and slowing, increase continually during stimulation until a practically steady maximum is reached if the stimulus is applied long enough; thus, smooth curves obtain (fig. 1A).

Infrequently there are slight irregularities in the ascending part of the curve, but they are practically negligible.

The subsidence of the reflex responses after cessation of the stimulus, on the other hand, presents frequently "dips" (fig. 6B) which are decidedly beyond experimental error. The significance of these irregularities will be taken up in the discussion.

Direct stimulation of either the vagi or the accelerators yields invariably smooth curves (fig. 1B).

B. *Rebound.* Intimately related with the irregularities of the subsidence of the reflex responses is the fact that the response may sometimes change its sign after the stimulus ceases—i.e., an acceleration is succeeded by a slowing. We may denote such a change in sign by the term "rebound" in Sherrington's sense (cf. McDowall, 1931), although the situation is more complex than in the case of spinal reflexes (see discussion). Figure 2A illustrates a typical case of rebound on central stimulation of the sciatic when the heart is normally innervated, succeeded (fig. 2B) by an irregular subsidence of the acceleration, without rebound, after removal of the stellates and section of the left vagus.

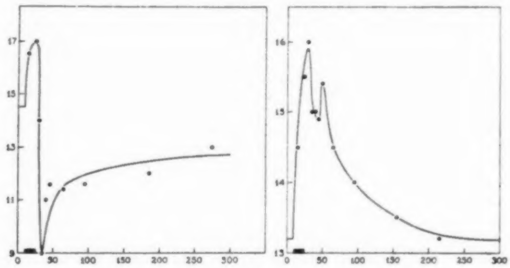


Fig. 2a Fig. 2b

Fig. 2A. Rebound on central stimulation of the sciatic; heart normally innervated. Frequency: 20 per second. Ordinates: heart rate per 5 seconds. Abscissae: time in seconds.

B. Disappearance of the rebound after section of the left vagus and removal of the stellate ganglia in the same animal.

C. *After-discharge and subsidence of central inhibition.* The recovery of the basal heart rate after direct stimulation of the vagus, even sufficient to stop the heart for several seconds, never exceeds an interval of 15 seconds. Reflex slowing of the heart through the vagus, on the other hand, persists usually for several minutes (up to about 10; see figs. 2 and 6). It will be shown in the discussion that this must necessarily be interpreted as a prolonged after-discharge from the reflex center (cf. McDowall, *loc. cit.*).

The time necessary for the heart to attain practically its maximum slowing on direct stimulation of the vagus is again relatively short (usually about 10 seconds). The subsidence of reflex acceleration produced by inhibition of the vagal center, however, is longer (again several minutes). The disappearance of central inhibition, therefore, is likewise strikingly slow.

As regards the accelerators the maximum response is rapidly obtained on direct stimulation (from 10 to 15 seconds), while the subsidence of reflex inhibition of the accelerator center is long (up to 15 minutes). There is not such a sharp contrast between direct and reflex stimulation of the accelerators, because the recovery from the direct response may last for several minutes (up to 6).

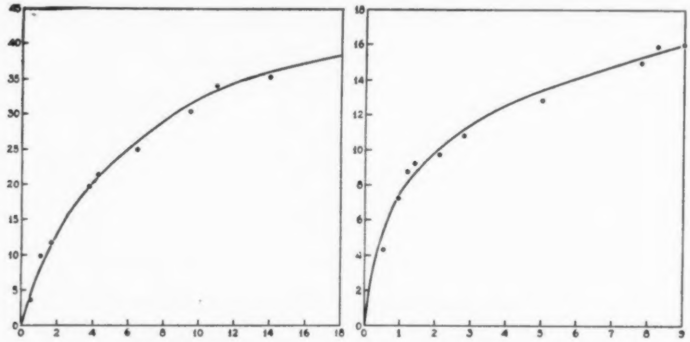


Fig. 3a Fig. 3b

Fig. 3A. Reflex excitation of the right vagus as a function of the frequency of maximal afferent stimulation of the left vagus and depressor cut peripherally; accelerators removed. Ordinates: maximal per cent slowing of the heart. Abscissae: frequencies per second.

B. Reflex inhibition of the accelerators as a function of the frequency of maximal afferent stimulation of the left vagus and depressor cut peripherally; right vagus cut, accelerators intact. Ordinates: maximal per cent slowing of the heart. Abscissae: frequencies per second.

D. *Influence of the frequency of afferent stimulation on the maximum of the reflex responses.* As stated previously (section A), if the stimulus is sustained long enough (usually beyond 15 seconds) the reflex responses reach a steady maximum which persists until the onset of fatigue (about 1 minute). This maximum, as in the case of direct stimulation of the cardiac nerves (Rosenblueth, 1932), is a continuous function of the frequency of stimulation. Figure 3 illustrates this function. Curve A is a typical example of central excitation, and curve B of inhibition. All the cases studied yielded similar results.

Cardiac reflexes involve reciprocal innervation (Rosenblueth and Freeman, 1931). If the nerve supply to the heart is intact, both the vagi and the accelerators will therefore participate in the reflex responses. If a curve like those in figure 3 is constructed from such a preparation and either set of nerves is then severed and a new series of observations made, the difference between the original responses and those now obtained should represent the part corresponding to the nerves abolished. This was found to be the case, for the differences plotted against frequency furnish curves similar to those obtained directly.

E. *The relations between frequency of afferent stimulation and efferent discharge.* The curves plotted in figure 3 show that the responses, and there-

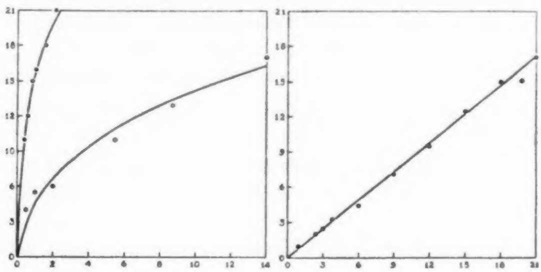


Fig. 4a Fig. 4b

Fig. 4. Reflex excitation as a function of input.

A. Lower curve (circles): left vagus and depressor stimulated centrally; right vagus intact; accelerators removed. Upper curve (dots): right vagus stimulated peripherally in the same preparation. Ordinates: slowing of the heart per 10 seconds. Abscissae: frequencies per second.

B. Straight line obtained by comparing the frequencies which give rise to identical responses in the two experimental curves of which only parts are reproduced in A. The frequencies were read after plotting the curves in A at suitable scales to insure greater accuracy. Ordinates: efferent rate. Abscissae: afferent rate.

fore the efferent discharges, are continuous functions of the input (rate of afferent stimulation). Any given response is indicative of a given number of efferent nerve impulses per unit time, whatever the number of nerve fibers concerned (Rosenblueth and Rioch, *loc. cit.*). The relations between input and output may therefore be established by plotting the afferent frequency necessary to obtain a given reflex response against the frequency of direct maximal stimulation of the efferent nerve which will insure an identical heart rate in the same preparation (figs. 4A and 5A). This method applied to reflex central excitation of either the vagus or the accelerators yielded invariably a very satisfactory linear relationship as exemplified in figure 4B. In the case of central inhibition the curves obtained were always of the type illustrated in figure 5B. This curve shows

the degree of inhibition of the tonic activity in the center, obtained by the different frequencies of afferent stimulation, in terms of the decrease in the rate of efferent discharge.

F. *The influence of frequency of afferent stimulation on the subsidence of the reflex responses.* The subsidence is slower the higher the frequency of afferent stimulation, both for central excitation and inhibition. Figure 6A exemplifies typical results. When the curves of subsidence present dips, these dips occur later and are more marked for the higher than for the lower frequencies (fig. 6B).

G. *Mathematical analysis.* The curves of subsidence of the responses, when smooth, fit approximately a simple exponential function of time, as revealed by semi-logarithmic plotting. This is true both for central excitation and inhibition. The rate of decay of both processes is therefore proportional to the value present at a given moment.

The curves illustrated in figure 3 fit adequately hyperbolas of the form $y = \frac{x}{k + k'x}$. Since the corresponding curves for direct stimulation of either the vagi or the accelerators belong to the same family of hyperbolas, the straight lines exemplified in figure 4B are readily explained (see Rosenblueth and Rioch, *loc. cit.*).

The curves illustrated in figure 5B are also hyperbolas of the same general equation.

Discussion. The interest of the data reported lies primarily in the bearing they have on the nature of central excitation and inhibition. Before this bearing is examined, however, the following considerations should be mentioned.

The sciatic and the left vagus possess afferents capable of inhibiting and exciting both the vagus and the accelerators. The depressor has been often considered a purely cardio-inhibitory nerve, but, since Bayliss (1908) found a reversal of its action after strychnine, it probably contains excitatory fibers (Bremer, 1922, 1925). The responses recorded in all cases were therefore the resultant of simultaneous central excitation and inhibition of the two efferents. It is reasonable, however, to assume that for any given frequency of maximal afferent stimulation an equilibrium will ensue during which either excitation or inhibition will be dominant. This assumption is corroborated by the plateau at the maximum of the response (fig. 1). It is likewise reasonable to expect that the relative proportions of central excitation and inhibition for any afferent nerve in a given preparation are constant, independent of the frequency of stimulation. This expectation is confirmed by the similarity between the curves of the responses to different frequencies (fig. 6) and by the continual ascent of the curves correlating the maximum of the responses with the corresponding frequencies (fig. 3).

The irregularities in the curves picturing the subsidence of the responses (fig. 6B) may be due to differences in the time-course of the dissipation of central excitation and inhibition, since the response is a resultant of the two simultaneous antagonistic processes. The dual innervation of the heart may also play a rôle. In some cases, however, and particularly in the phenomenon of rebound (fig. 2A), the "circulatory proprioceptors" undoubtedly are largely responsible for the changes in heart rate. The term circulatory proprioceptors is here used to denote afferents such as the depressor, Hering's nerves and possibly others, which arise from the vessels and whose stimulation modifies the heart rate reflexly.

In all the cases studied the proprioceptors tend to decrease the cardiac response, acceleration or inhibition, by exerting an influence in the opposite direction, since the changes of heart rate and blood pressure bear the same sign; i.e., the reflex slowing of the heart is associated with a fall in blood pressure which will give rise to proprioceptive afferent impulses tending to accelerate the heart, and the reflex acceleration *vice versa*. The long subsidence-time of the reflex responses is therefore particularly significant; if total deafferentation of the circulatory system were performed the responses might be even longer.

In the following discussion of central excitation and inhibition the assumption will be made that each of these processes is identical in nature in all neurones. Until proof to the contrary is forthcoming this assumption is legitimate. A detailed examination of all the theories which have been proposed to explain central excitation and inhibition is beyond the scope of this paper. The deficiencies of some of these theories have been pointed out by Eccles and Sherrington (1931b and c).

After-discharge has been explained by Forbes (1922) as due to a continued bombardment of the neurones of the final common path by impulses arriving from "delay paths." Sherrington, who was previously reluctant to accept this explanation (1925), has later endorsed it (Eccles and Sherrington, 1931a). Fulton's (1926) objection, that after-discharge in spinal reflexes may be too long (5 or 6 seconds) to be plausibly explained by delay paths, has been met by the theory of self-reëxciting, reverberating chains (Ranson and Hinsey, 1930; Forbes, Davis and Lambert, 1930; Bárány, 1932; Lorente de Nó, 1932). We are here, however, dealing with after-discharges which may last 10 minutes, a period much too long to be easily explained by neurone detours, and we feel, therefore, disinclined to accept that hypothesis until direct proof shall be furnished that such mechanisms exist.

The continuity of the curves of subsidence and their similarity for different frequencies of stimulation in a given preparation (fig. 6) constitute further objections to the theory of reverberating delay paths. This subsidence is probably not due to fatigue, for the full response may be regained

at any time by renewing the stimulus. It is not due to antagonistic proprioceptive impulses, for if it were so, total deafferentation would allow a perpetual response, which is absurd. It would then have to be due to fortuitous impulses silencing successively the different reverberating chains involved; this is improbable because of the similarity of the curves obtained. The continuity of the curves (lack of steps) requires a larger number of chains than it seems plausible to imagine.

Finally, the fact that after-discharge is a continuous function of the frequency of stimulation (fig. 6) is likewise a serious argument against the probability of the existence of reverberating paths. Since the afferent stimuli used were always maximal, the only way to explain longer after-discharge with higher frequencies would be to assume as large a number of chains with systematically graded thresholds, recruited at different frequencies, as would be necessary to explain the continuity of the subsidence. Practically the whole system of reverberating chains would have to belong to the subliminal fringe; otherwise, if all the chains responded to a single afferent stimulus, subsidence would be independent of the frequency of stimulation—i.e., the duration of after-discharge would always be the same.

These elaborate assumptions, necessary to explain the subsidence of the reflex responses in terms of reverberating delay paths, make the hypothesis quite improbable. We conclude, therefore, that after-discharge is better explained as a persisting supraliminal c.e.s. (Sherrington). In rejecting the delay paths we automatically reject the interpretations furnished by Eccles and Sherrington for central excitation (1931b) and inhibition (1931c), since their theories postulate this continuous bombardment in order to explain after-effects.

There are other objections to be made to Eccles and Sherrington's theories. If the excitatory process in the perikaryon were identical with that of peripheral nerve, any substance capable of stimulating the former should likewise be capable of exciting the latter, and *vice versa*. Such may not be the case, however, for ergotoxine, for instance, produces direct central excitation of the motoneurones (Rosenblueth and B. Cannon, 1933), but is apparently incapable of stimulating peripheral nerve fibers, since its motor effects disappear on section of the nerves, even if large doses (e.g., 10 mgm. per kgm.) are administered. The specific effects of other drugs (e.g., nicotine) are also interesting in this respect. This argument is only stated tentatively, however, for it would be necessary to establish qualitative differential effects before a verdict could be passed.

The main objection to accepting any physical explanation of c.e.s. and c.i.s. lies in the difficulties which arise when correlation of the two opposed processes is attempted. The nerve impulses impinging on the surface of a neurone are all identical in nature. It is to be expected, therefore, that the physical surface phenomena produced by these nerve impulses will

always be the same. We fail to conceive how some nerve impulses depolarize the surface, producing excitation, while others stabilize it, giving rise to inhibition (Sherrington, 1929).

We are thus led to examine another view, that of the chemical nature of central excitation and inhibition (Sherrington, 1925; Ballif, Fulton and Liddell, 1925; Fulton, 1926; Samojloff and Kisseleff, 1927). It is interesting to note that this theory does not postulate any process different from those occurring in peripheral nerve-muscle preparations (cf. Lucas, 1917), since the transmission of the nervous impulses to smooth muscles is me-

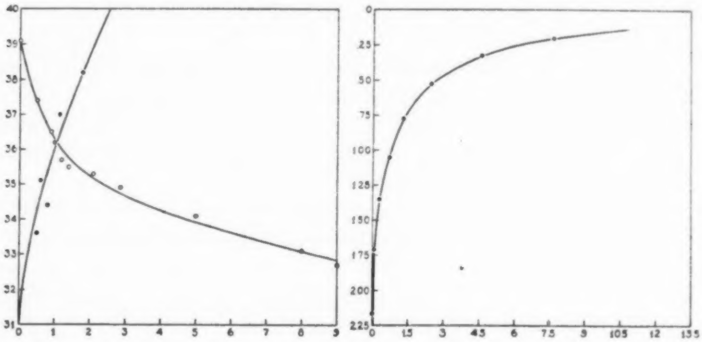


Fig. 5a Fig. 5b

Fig. 5. Reflex inhibition as a function of input.

A. Descending curve (circles): left vagus and depressor stimulated centrally; right vagus cut; accelerators intact. Ascending curve (dots): right accelerator stimulated peripherally in the same preparation. Ordinates: heart rate per 10 seconds. Abscissae: frequencies per second.

B. Curve obtained by comparing the frequencies which give rise to identical heart rates in the two experimental curves of which a part is reproduced in A. The readings were done after plotting at suitable scales to insure greater accuracy. Ordinates: efferent rate. Abscissae: afferent rate.

diated chemically (see Cannon, 1933, for references). It is well known that chemical substances may excite or inhibit specifically certain neurones (cf. above, the effects of ergotoxine). The retina is especially noteworthy since it is a structure which belongs to the central nervous system. The verdict is practically unanimous (see Hecht, 1929) that physiological excitation occurs at a given concentration of a chemical substance.

The sole argument opposed by Eccles and Sherrington (1931b) to the chemical theory is the following. Because an antidromic impulse apparently removes c.e.s. in a motoneurone, c.e.s. must be restricted to those parts of the motoneurone accessible to such an impulse—i.e., by analogy with peripheral nerve, the surface membrane of the motoneurone and its

dendrites. C.e.s. would then be confined to this surface membrane. A chemical substance is unlikely to be restricted to a surface or to be removed by antidromic impulses or reflex discharges.

It is, however, possible that the analogy assumed is unjustified. Antidromic impulses, on whose effects the argument is primarily based, valuable though they are as blocking agents for certain efferent impulses (see Creed, Denny-Brown, Eccles, Liddell and Sherrington, 1932), may be but artefacts for the analysis of central excitation and inhibition. Furthermore, the excitatory and inhibitory chemical substances might easily be restricted to the superficial layer of the neurone, just as the ions, on the presence of which the polarization of the membrane depends, are restricted to the superficial layer.

The linear relationship between input and output (fig. 4B) for central excitation does not convey any information as to the nature of c.e.s. but is compatible with any of the theories examined. It is nevertheless interesting that the output is directly proportional to the input, notwithstanding the complexity of the systems involved.

The consistent relations between input and degree of inhibition of a steady tonic excitation (fig. 5B) confirm the "quantitative cancellation" of c.e.s. by c.i.s. (Eccles and Sherrington, 1931c), but there is no "true algebraic summation," since a straight line does not obtain. The experimental curves might be explained by a chemical combination between the inhibitory and the excitatory substances, but they do not exclude other processes.

From the foregoing data and arguments the most adequate theory for central excitation and inhibition appears to be the following. Nerve impulses impinging on a neurone give rise to quanta of excitatory (c.e.s.) or inhibitory (c.i.s.) substances, according to the differentiated structures within the cell on which they act. Both c.e.s. and c.i.s. are destroyed at a rate proportional to the concentration. For a steady input and at equilibrium the concentrations of c.e.s. and c.i.s. are proportional to the rate of bombardment of the neurone by nerve impulses. C.e.s. attains supraliminal values; this explains after-discharge. The rate of discharge of impulses by the neurone is proportional to the concentration of c.e.s. The output from a center is therefore proportional to the excitatory input. C.i.s. combines with c.e.s., inactivating the latter.

Our attention has been drawn to a recent paper by A. W. Kibjakow (Pflüger's Arch., 1933, *232*, 432) which demonstrates a chemical transmission of preganglionic to postganglionic nerve impulses in sympathetic ganglia.

SUMMARY

Reflex changes of heart rate on afferent maximal stimulation at varying frequencies of the depressor, the left vagus and the sciatic were recorded

from cats with either the vagi or the accelerators severed. These responses were compared with those obtained from peripheral maximal stimulation at varying frequencies of either the right vagus or the right accelerators.

Methods are described by which the time-course of the responses may be followed (section A and figs. 1, 2 and 6).

The subsidence of central excitation and inhibition in these reflexes may be very long (up to 10 minutes).

The maximum of the reflex responses at equilibrium is a continuous function of the frequency of afferent stimulation (section D and fig. 3).

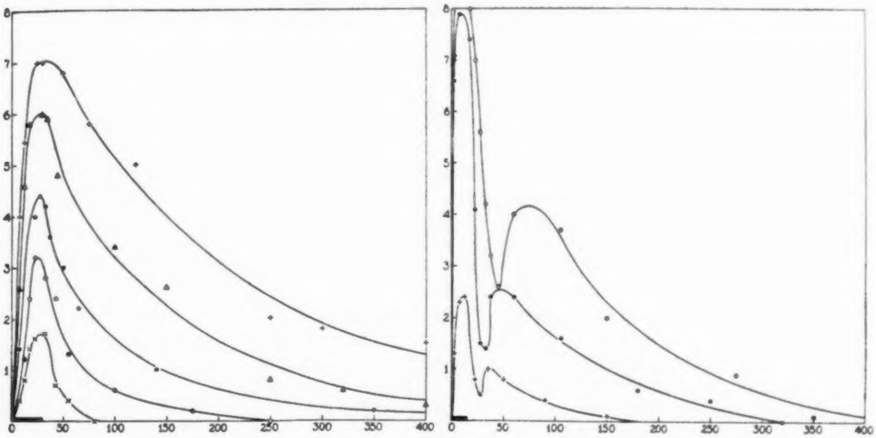


Fig. 6a Fig. 6b

Fig. 6. Subsidence of reflex responses as a function of the rate of afferent stimulation.

A. Left vagus and depressor stimulated centrally; right vagus cut; accelerators intact. Ordinates: slowing of the heart per 10 seconds. Abscissae: time in seconds. Frequencies of stimulation: 0.95; 1.5; 3.0; 7.8; and 20.0 per second.

B. As in A, but right vagus intact. Ordinates: slowing of the heart per 5 seconds. Abscissae: time in seconds. Frequencies of stimulation: 1.9; 6.0; and 22.0 per second. The top of the last curve during stimulation is omitted in the figure.

The reflex output of the centers is directly proportional to the excitatory input (fig. 4). For a given degree of tonic activity, the output is a continuous, smooth, non-linear function of the inhibitory input (fig. 5).

The duration of the subsidence of the reflex responses is a continuous function of the afferent frequency (fig. 6).

After-discharge is discussed (p. 299). It is concluded that reverberating delay paths do not explain it satisfactorily.

Some objections to Eccles and Sherrington's (1931) theories for central excitation and inhibition are presented (p. 300). It is concluded that a chemical hypothesis explains the data more adequately (p. 302).

REFERENCES

BALLIF, L., J. F. FULTON AND E. G. T. LIDDELL. 1925. Proc. Roy. Soc., B, **98**, 589.

BÁRÁNY, R. 1932. Deutsch. Zeitschr. f. Nervenh., **124**, 216.

BAYLISS, W. M. 1908. Proc. Roy. Soc., B, **80**, 339.

BISHOP, G. H. AND P. HEINBECKER. 1932. This Journal, **100**, 519.

BREMER, F. 1922. Compt. Rend. Soc. Biol., **87**, 1055.

1925. Arch. Int. Physiol., **25**, 131.

CANNON, W. B. 1933. Science, **78**, 43.

CREED, R. S., D. DENNY-BROWN, J. C. ECCLES, E. G. T. LIDDELL AND C. S. SHERRINGTON. 1932. Reflex activity of the spinal cord. Oxford.

ECCLES, J. C. 1931. Proc. Roy. Soc., B, **107**, 557.

ECCLES, J. C. AND C. S. SHERRINGTON. 1931a. Ibid., **107**, 586.

1931b. Ibid., **107**, 597.

1931c. Ibid., **109**, 91.

FORBES, A. 1922. Physiol. Rev., **2**, 361.

FORBES, A., H. DAVIS AND E. LAMBERT. 1930. This Journal, **95**, 142.

FULTON, J. F. 1926. Muscular contraction and the reflex control of movement. Baltimore.

HECHT, S. 1929. The foundations of experimental psychology. Worcester.

LORENTE DE NÓ, R. 1932. Trans. Amer. Otol. Soc., **22**, 287.

LUCAS, K. 1917. The conduction of the nervous impulse. London.

MCDOWALL, R. J. S. 1931. Journ. Physiol., **71**, 612.

RANSON, S. W. AND J. C. HINSEY. 1930. This Journal, **94**, 472.

ROSENBLUETH, A. 1932. Ibid., **102**, 12.

ROSENBLUETH, A. AND B. CANNON. 1933. Ibid., **105**, 373.

ROSENBLUETH, A. AND N. E. FREEMAN. 1931. Ibid., **98**, 430.

ROSENBLUETH, A. AND D. MCK. RIOCH. 1933. Ibid., **104**, 519.

SAMOJLOFF, A. AND M. KISSELEFF. 1927. Pflüger's Arch., **215**, 699.

SHERRINGTON, C. S. 1925. Proc. Roy. Soc., B, **97**, 519.

1929. Ibid., **105**, 332.

AN AMPLIFIER, RECORDING SYSTEM, AND STIMULATING DEVICES FOR THE STUDY OF CEREBRAL ACTION CURRENTS[1]

E. L. GARCEAU AND H. DAVIS

From the Department of Physiology, Harvard Medical School

Received for publication October 9, 1933

In the course of a preliminary survey of action currents in the central nervous system, and particularly the auditory pathways (Saul and Davis, 1932), it soon became evident that adequate study of these phenomena could be carried out only with instruments of greater sensitivity and resolving power than the amplifier and string galvanometer employed in the original experiments. We therefore undertook to design and construct an amplifier competent to deal with transient signals of the order of 1 or 2 microvolts and powerful enough to operate a cathode ray oscillograph. For study of auditory phenomena the generation and measurement of pure tones appeared essential and suitable apparatus for this purpose was likewise constructed.

The electrical apparatus is all mounted upon relay racks of the standard 19 inch width. The five bays used are mounted on small trucks so that they can be moved from place to place by one man. The bays are in order from left to right (1) a low level input amplifier; (2) a bay for input accessories, as yet not fully equipped; (3) an oscillograph bay, mounting a cathode ray oscillograph tube, camera and various patching panels; (4) the stimulator bay, containing apparatus for generation of both electrical and auditory stimuli, and (5) a bay for the high level amplifier which drives the oscillograph and also the output loud-speaker. The essentials of the wiring diagrams of these bays are represented in figure 1.

The electrodes which are used to pick up the action potentials are of the familiar Adrian-Bronk coaxial type (Adrian and Bronk, 1929). They are made of hypodermic needles with an insulated silver or copper core, the end of which is flush with the bevel of the needle. The electrodes are connected to the selector switch of the first amplifier by means of flexible

[1] The rather considerable expenses of constructing the apparatus described in this paper have been defrayed by grants to Dr. L. J. Saul of the Department of Psychiatry from the DeLamar Mobile Research Fund of the Harvard Medical School, and by other grants from the Josiah Macy, Jr. Foundation, from the American Otological Society and from several anonymous donors. To all of these we express our grateful acknowledgment.

shielded cords. The selector switch is capable of connecting the grid of the first tube to any one of four separate electrodes, all of which may be placed in the tissue at the same time. The selector switch is also connected to a calibrator which will put from 2 to 1000 measured microvolts on the grid of the first tube for standardizing purposes. A standard alternating signal of any frequency, as well as a direct current signal, may be used for checking the over-all gain of the amplifier. We have hopes of introducing

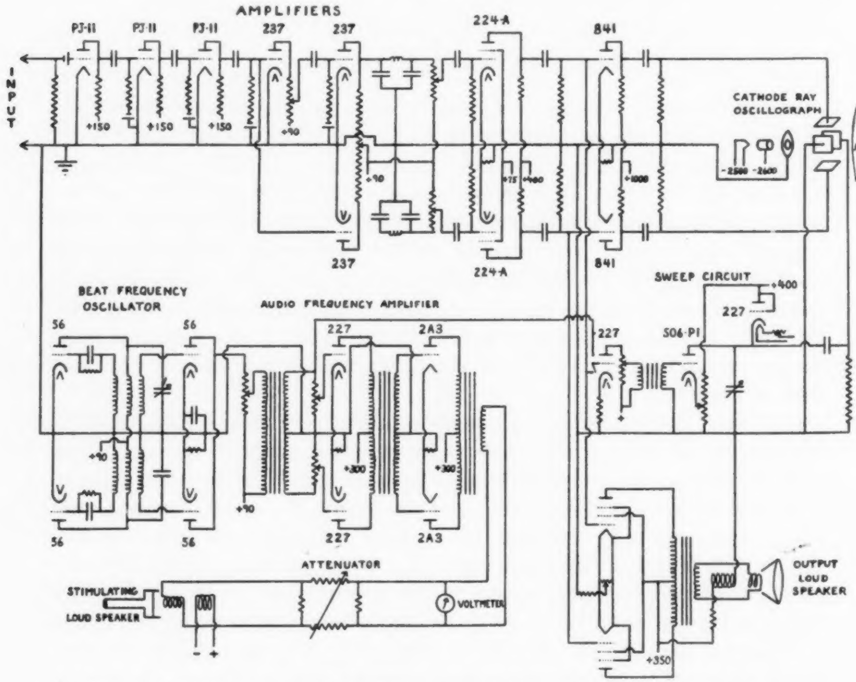


Fig. 1. Wiring diagram of amplifiers, stimulators and accessory apparatus

at this point a low level transformer covering a wide frequency range which will increase the sensitivity below the present noise level, which is about 1 microvolt.

The first three stages of the amplifier consist of type P. J. 11 General Electric Company pliotrons. These stages are all resistance-coupled. It has been found necessary to use plate and grid resistors which are wire-wound and coupling condensers of mica in order to reduce noise to the absolute minimum. The tubes are extremely microphonic and have, therefore, been very carefully mounted. Small sockets are attached to the tube

base and the assembly is wrapped in cotton and placed in a heavy covered lead pot whose walls are half an inch thick. The pots are suspended on long helical springs, and each is mounted in a separate compartment of a steel case. The case is surrounded by one inch of acoustic felt and mounted in another case which is attached to the relay rack. The rack is well weighted down at the bottom by 300 pounds of batteries. This system makes the input amplifier completely free from microphonic disturbances. The P. J. 11 tubes are heated by a 6 volt lead battery and their plates are supplied by a 150 volt lead battery.

Above these three stages is an impedance-reducing stage using type 237 tubes. Here also is a phase-splitting stage which supplies no amplification but which delivers the output in push-pull (with grounded neutral) to the succeeding amplifiers. These tubes are run from the same A battery as the first three stages but have a separate B battery. In the topmost panel above these two stages is a 2000 cycle cut-off low-pass filter which may be used if desired to cut out the high-frequency components of the Schott effect in the tubes. Here also is a balanced attenuator to control the effective gain of the entire amplifier.

The output of the first amplifier bay is led to a voltage amplifier at the top of the fifth bay. This bay, and in fact all of the apparatus in the third, fourth and fifth bays, derives its power solely from the 115 volt 60 cycle single phase lines. Plate supply rectifiers and filters are built into each of the alternating current operated panels and are all independent. The voltage amplifier uses two 224A screen grid tubes in series. Inasmuch as there is no provision in each stage for cancelling the even harmonics produced by amplifier distortion, the amplifier can hardly be said to have a true push-pull circuit, but nevertheless many advantages are derived from using two tubes in series. It is possible to handle twice the signal voltage which a single tube would take and also to balance out a good deal of hum in the power supply. The 224A's are resistance-coupled and their output feeds in parallel the amplifier for the loud speaker and the amplifier for the cathode ray oscillograph, and also may be used to synchronize the cathode ray time-axis with any dominant frequency in the signal.

The bottom panel of the fifth bay is a high voltage power amplifier which drives the oscillograph. Type 841 tubes are used with a 1000 volt plate supply. The second panel from the bottom is a low voltage rectifier to supply filament current to the 841 tubes. The total voltage gain up to this point can be as high as 100,000,000. The time-constant of each of the six stages is 6 seconds. The over-all time constant is naturally less, and we find by experiment that the deflection from a constant applied voltage falls to half its value in about five-eighths of a second. A small series input condenser can be used to prevent blocking of amplifier by slow large potentials from shifting contacts, etc. This further reduces the time-constant

of the amplifier as a whole, but introduces no appreciable distortion of the spikes of action potentials or any significant reduction of alternating current signals of frequencies above 150 cycles per second.

The third panel from the bottom in the 5th bay contains a dynamic loud speaker which is operated by two type 247 pentodes in true push-pull. This loud speaker is used for listening to the quality of the signal. These tubes may also be used to drive a DuBois oscillograph.

The fourth panel from the bottom contains a circuit which supplies a linear time axis for the oscillograph. This consists of the familiar arrangement of a condenser which charges at a nearly linear rate and which is periodically discharged almost instantaneously through a thyratron, whose grid is tripped at a rate corresponding to a sub-multiple of any dominant signal frequency in the input. This circuit may alternatively be controlled

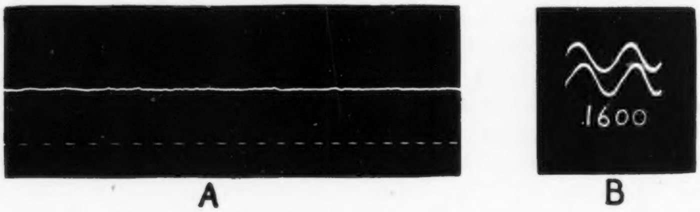


Fig. 2. A. Base line, input leads shorted, sensitivity 200 mm. on film per millivolt. Time marker 5σ. Low pass filter out.

B. Standing waves, 1600 cycles per second. Upper, electrical calibrating wave; lower, sound wave recorded through condenser microphone. Sensitivity, 65 mm. on film per millivolt.

by impulses derived directly from the electric or the acoustic stimulators when these are being used.

Above this is a power supply unit of the General Radio Company which supplies the necessary voltages for the cathode ray tube.

The fourth bay from the left contains at the top a special multivibrator stimulator to be described in more detail elsewhere, which delivers rectangular waves adjustable as to strength, duration and frequency for electrical stimulation. The fifth panel from the top of this bay contains a thyratron oscillator which delivers very sharply peaked waves for electrical and auditory stimulation. The rest of the panels comprise an audio frequency signal-generating system. A General Radio beat frequency oscillator, converted to work with a.c. power supply, provides frequencies from about 30 to 10,000 c.p.s. The signals are amplified by a two-stage push-pull transformer-coupled amplifier using 2A3 triods in the output. About 10 watts maximum signal is available. The output is measured by a small rectifier voltmeter and then passes through a three step adjustable

pi attenuator of 120 db total insertion loss to a dynamic horn type loud-speaker. The details of the remainder of the acoustic system will be described in the succeeding paper (Davis, Derbyshire, Lurie and Saul).

A small calibrated condenser microphone is suitably mounted in a situation closely corresponding to the animal's ear to receive the sound waves from the tube conveying them from the loud speaker. The controls for the amplifier of the microphone are located in the panel at the top of the second bay. The output of this amplifier is suitably attenuated and may be introduced into the first stage of the low level amplifier, thus giving a comparison between the wave shapes of the sound and the responses from the cat. These stimulating sound waves to the cat's ear may be calibrated in terms of dynes per square centimeter through the condenser microphone and its amplifier by means of the measured alternating current output from the oscillator. The second bay also contains a capacity bridge designed to remove the artefact produced by electrical stimulation from the record of resulting action currents (cf. Bishop, 1927).

The central bay contains a Dumont cathode ray oscillograph tube which is mounted in a heavy iron case to reduce interference from magnetic fields. The camera may be swung up in front of the oscillograph where it automatically comes into the correct focus. It may be used to take still pictures, reduced in size in 4:1 ratio, of standing wave forms when the synchronized time axis is used. It may also be used to take continuous records by driving the film with an electric motor. When this is done a switch is operated to disconnect the time axis from the cathode ray tube and to apply the signal to give only horizontal deflections of the spot. For time marker a small beam of light interrupted by a synchronous clock motor is reflected on to the edge of the moving film. Small dots are thus produced on the film, giving time intervals of 5 σ.

The entire apparatus is housed in a small electrically shielded room painted black on the inside and sound-proofed. The animal preparation is mounted in a smaller room within the main room which is also shielded electrically and acoustically.

Figure 2 illustrates several types of record taken with this apparatus, including A, baseline of amplifier with input lead short-circuited, sensitivity 200 mm. on film per millivolt; B, 1600 cycle sine waves, one an electrical calibrating wave, the other a sound wave recorded through the condenser microphone, illustrating degree of linearity of time axis. Physiological records illustrating other aspects of its performance will be found in the succeeding paper (Davis, Derbyshire, Lurie and Saul).

This apparatus has been gradually developed during a period of a year and a half, being modified in many details from the original plans as a result of experience. It is now quite reliable in operation, and although employing 47 vacuum tubes, it has proved by no means too elaborate for the

study of the electrical responses of the cochlea and the action currents of the auditory pathways.

REFERENCES

Adrian, E. D. and D. W. Bronk. 1929. Journ. Physiol., **67,** 119.
Bishop, G. H. 1927. This Journal, **72,** 462.
Davis, H., A. J. Derbyshire, M. H. Lurie and L. J. Saul. This Journal, cvi, 311.
Saul, L. J. and H. Davis. 1932. Arch. Neurol. and Psychiat., **28,** 1104.

THE ELECTRIC RESPONSE OF THE COCHLEA

H. DAVIS, A. J. DERBYSHIRE, M. H. LURIE AND L. J. SAUL[1]

From the Departments of Physiology, Otology and Laryngology, and Psychiatry, Harvard Medical School

Received for publication October 9, 1933

Fresh impetus to study of the auditory mechanism was given by the experiments of Wever and Bray (1930), who listened to the amplified electric responses picked up from the auditory nerve and found that tones and even spoken words used as stimuli could be easily recognized. Although auditory action currents were already familiar (Forbes, Miller and O'Connor, 1927), it seems in the light of subsequent analysis that this was probably the first occasion on which the electric response of the cochlea itself was detected. The dual nature of the response was not immediately recognized, and the first efforts were directed primarily to proving that the phenomenon was genuinely biological in nature and not an instrumental artefact or dependent essentially on the physical properties of electrodes and tissues. Adrian (1931) suggested a "microphonic action of the cochlea," and Saul and Davis (1932) emphasized the distinction between true action currents in the auditory pathways and an electric response emanating apparently from the inner ear. The present paper deals with the characteristics of this latter type of response and its relation to the stimulating sound waves.

APPARATUS AND METHODS. The apparatus employed in these experiments consists primarily of a cathode ray oscillograph combined with a loud speaker and activated through suitable amplifiers,[2] and is described in detail in a separate communication (Garceau and Davis, in press). This combination allows us to see and hear the responses simultaneously. A condenser microphone makes possible measurement of intensity and study of the form of the sound waves used as stimuli. Moderately pure electrical waves of various frequencies, generated by means of a beat frequency oscillator, are amplified, measured and appropriately attenuated if necessary, and then are finally transformed into sound by a loud speaker.

[1] Commonwealth Fund Fellow in Psychiatry, Boston Psychopathic Hospital and Harvard Medical School.

[2] The expenses of constructing this apparatus have been defrayed by grants from the DeLamar Mobile Research Fund of the Harvard Medical School, from the Josiah Macy, Jr. Foundation, from the American Otological Society and from several anonymous donors. To all of these we express our grateful acknowledgment.

The sound is conveyed to one or both ears of the animal through large rubber tubes. In order to minimize resonance and distortion certain precautions are observed. The loud speaker selected is one designed to operate with approximately the load which this system places upon it. The single tube leading from the loud speaker divides in a Y joint to two smaller tubes, the sum of whose cross sectional areas (150 mm.^{2}) is approximately equal to that of the single original tube. Small side tubes lead out from these to fit snugly with rubber-cushioned joints into metal ear specula which are sewed into the external auditory canals of the animal. The side tubes can be closed by stopcocks, but the bore (5 mm.) of the side tubes with stopcocks open is uniform throughout and is small relative to that of the main tubes. The main tubes continue for several feet beyond the side tubes and are packed first lightly then more snugly with cotton to approximate acoustically tubes of infinite length. Opposite one of the side openings to the cat is a similar opening which leads directly to a condenser microphone 20 mm. in diameter. Figure 1, showing the form of the electrical wave delivered to the loud speaker and of the sound wave as recorded by the condenser microphone, illustrates the degree of purity of the waves delivered by this system. The loud speaker generating the stimulating tone is mounted in a sound-proof box suspended directly beneath the animal table. The animal is mounted in a small sound-deadened and electrically shielded room within a larger shielded room housing the amplifiers and other electrical apparatus.

Throughout the experiments reported in this paper, electrical contact with the animal was made by means of a silver plate laid upon the muscles of the back of the neck and a differentiated electrode usually placed in contact with the round window. The differentiated electrode consisted of a fine silver wire electrically shielded except for the last 3 cm., ending in a small coil carrying a short bit of cotton thread soaked in Ringer's solution. It was this wick which actually made contact with the round window.

Cats under avertin anesthesia were used almost exclusively, although a few observations were also made on guinea pigs. The usual dosage of avertin was 220 mgm. per kilo, given intraperitoneally, which sufficed to produce a very satisfactory surgical anesthesia without obliterating the responses of the auditory pathways. In the course of a long experiment small additional doses were usually given to maintain an even level of anesthesia, usually such that a moderate degree of muscular tone was present. The round window was exposed through the bulla, which in turn was usually approached from the posterior and lateral aspect of the neck. In most experiments the cranium was also opened to make possible observations of action currents. It was established experimentally that the opening of the cranium with consequent fall of intracranial pressure did not

significantly affect the responses of the cochlea, with which we were immediately concerned.

CHARACTERISTICS OF THE ELECTRIC RESPONSE OF THE COCHLEA. Many of the characteristics of the electric response which can be recorded from the neighborhood of the cochlea have already been reported by Wever and Bray (1930), Adrian (1931), Adrian, Bronk and Phillips (1931), Hughson and Crowe (1932), Saul and Davis (1932), and Bast, Noer, West, Backus, Krasno and Eyster (1933), and we may say at once that our present results are in almost complete agreement with these reports when allowance is made for the dual character of the response which was recorded in many of the earlier experiments.

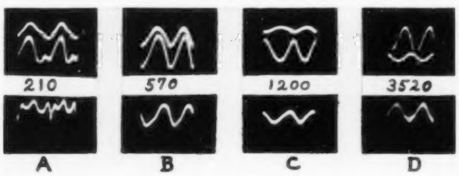


Fig. 1. (July 12, 1933.) Electrical wave (upper), sound wave (middle) as recorded by condenser microphone, and cochlear response (lower) at various frequencies.

A. Frequency 210, intensity 50 db. Note prominent harmonics which are more apparent than with weaker stimulation at the same frequency. Calibration curves are made at less than 50 db but do not change their wave form with change in intensity.

B. Frequency 570, intensity 31 db for all curves.

C. Frequency 1200, intensity 28 db for all curves. Sensitivity for A, B, and C 25 mm. on film per millivolt.

D. Frequency 3520, intensity 50 db, sensitivity increased to 88 mm. per millivolt. Calibration skips head amplifier, accounting for phase shift of 180°.

Form of the response and limits of frequency. Words spoken to the cat's ear are reproduced with great clarity and faithfulness. The speaker may be easily recognized by the quality of his voice. There is in addition a characteristic metallic quality, suggestive of a telephone or radio broadcast, which we shall show below is due to a relative accentuation of sound frequencies lying in the range between 800 and 2000 per second. For pure tones above 1000 per second reproduction of pure tones is faithful as to frequency and wave form, but diminishes progressively in intensity as the frequency is increased. We have detected a response visible on the cathode ray oscillograph and audible from the loud speaker up to slightly above 8000 per second in the cat and 7000 in the guinea pig, and have no reason to believe that this represents a physiological upper limit. It is probably a practical limit imposed by the power of our stimulating circuit and the sensitivity and resolving power of our recording apparatus. Figure 1

illustrates the wave forms of the responses corresponding to the stimulating waves shown in the same figure. A marked distortion of the lower tones, due to magnification of originally small harmonics, is clearly shown.

Localization. The round window is by far the most active spot which we have found. Other relatively good regions are the internal auditory meatus, the subarcuate fossa of the petrous bone within the cranial cavity, and the stapes. The response can also be detected from other parts of the temporal region and even from the surface of the cerebrum or cerebellum at more considerable distances. It behaves as though an electrical

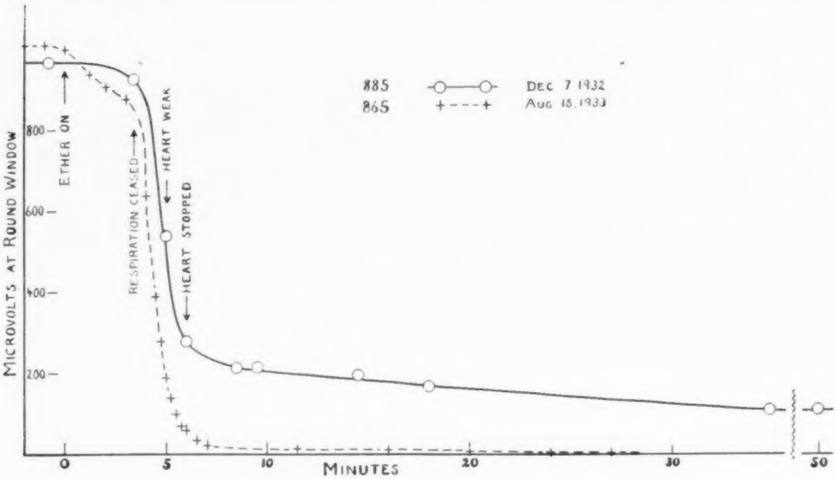


Fig. 2. (December 7, 1932 and August 18, 1933.) Cochlear response in microvolts peak voltage at round window, showing failure during etherization and death of animal. Frequency 865 and 885. Intensity approximately 40 db above one bar, maximal in each case. Heavy line is most typical curve, showing a slowly failing post-mortem response of about 150 microvolts. Notations as to failure of respiration and heart apply equally to both curves.

disturbance were generated in the region of the cochlea and could be detected at various distances, depending primarily on conditions of electrical conductivity and the relative position of the two electrodes with respect to the cochlea. It is partly on account of this localization that we refer to this phenomenon as "the electric response of the cochlea." In previous papers we have also referred to it as "spread" because it spreads diffusely through the tissues without reference to particular neural pathways.

Resistance to fatigue, anesthetics, mechanical damage and death. As was emphasized by the earlier investigators of this phenomenon, the response of the cochlea is highly resistant to various adverse conditions. At the

death of the animal the response falls rather rapidly to approximately 20 per cent of its previous intensity and may continue with slowly diminishing intensity for a period of one to several hours. We have studied it as long as 5 hours post-mortem, and it may persist even longer. In all such tests

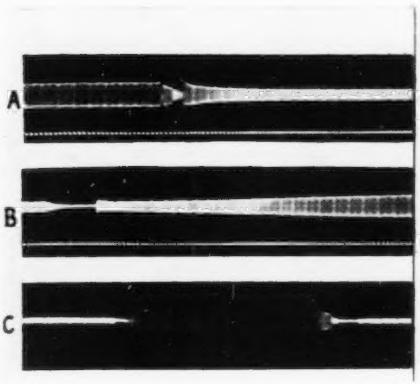


Fig. 3

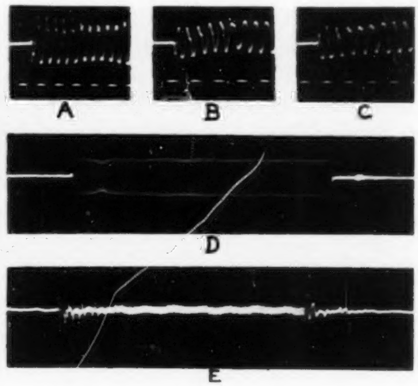


Fig. 4

Fig. 3. (August 3, 1933.) Cochlear response to supramaximal stimulation. Frequency 865. Sensitivity 15 mm. on film per millivolt.

A. Cochlear response. Increase in stimulation from 35 db to 55 db.

B. Same. 55 db to 35 db. Time marker equals 5σ.

C. Condenser microphone. 35 db to 55 db to 35 db.

During changes of intensity the stimulus ceases for from 20 to 50σ. The response to echoes in tube during this interval is clearly apparent.

Fig. 4. (July 26, 1933 and August 18, 1933.) Cochlear response to sudden onset and cessation of tone. Speed of film approximately the same in all cases.

A. Condenser microphone record. Frequency 428, intensity 41 db. Time marker 5σ. Half time of amplifier 0.63 second.

B. Round window response, beginning positive. Stimulation, etc., as in A.

C. Same as B. Response beginning negative. Note fast first cycle in round window response.

D. Condenser microphone. Frequency 2500, intensity 34 db. Duration of tone is 48σ.

E. Round window. Stimulation as in D. Note "on" and "off" effects. Disregard 60 cycle artefact.

In A and D the transient diminution in intensity shortly after the onset of the tone is due to echo effect in the sound tube.

great care was taken to rule out the possibility of instrumental artefacts, such as vibration of electrodes or electrical cross-talk between stimulating apparatus and the recording system. Some of these controls include the disappearance of the response with closure of the stopcock leading to the stimulated ear, its disappearance with interruption of electrical continuity

between electrode and round window, and the insensitivity of the electrode to vibration directly applied, as by touching its support with the handle of a vibrating tuning fork (cf. Davis and Saul, 1933).

Ether anesthesia does not greatly reduce the magnitude of the response until it is sufficiently profound to cause cessation of respiration or weakening of the heart beat (fig. 2). The application of novocaine crystals to the round window ultimately results in nearly complete abolition of the response.

Wever and Bray (1930c) and Adrian (1932) have pointed out the dependence of the response upon the integrity of the blood supply to the cochlea, and we also find that cutting the cochlear artery practically abolishes the response within 40 minutes, or at least causes it to fall promptly to the post-mortem level (fig. 2). Occasionally the first rapid fall has been followed by partial recovery and a second slower diminution. We have also seen the response fall smoothly to less than 0.5 per cent of its initial value within half an hour after death by etherization. The conditions determining these variations in "post-mortem" response have not yet been defined.

Puncturing the round window membrane with a needle or drilling through the petrous bone into the cavity of the cochlea and allowing escape of a little endolymph may cause any degree of reduction in the response from scarcely perceptible to about ninety per cent, depending on the extent of damage done, but the response is not abolished completely unless the basilar membrane is also injured. A very small puncture of the round window may actually increase the recorded response, due probably to the resulting diminution of electrical resistance.

The cochlear response seems to be practically immune to fatigue. Stimulation at intensities which are low or moderate, as judged by their loudness when received directly into our own ears, gives as large a response after many minutes of continuous stimulation as at the beginning. When still stronger stimuli, such as give rise to marked tinnitus and definite discomfort in the human ear are employed, we have occasionally observed a reduction of as much as 10 or 15 per cent in the response during the first few seconds of stimulation, followed by a maintenance of response at the new level. This phenomenon is best observed at frequencies between 700 and 1000 (fig. 3) and in some animals is difficult to demonstrate outside this range, at least with the intensities of stimulation at our disposal. It cannot be attributed to reflex contraction of the tensor tympani or the stapedius, as the latency of the beginning of diminution is not more than 2 σ. The condenser microphone shows that the intensity of stimulation remains constant while the response falls. We have as yet no explanation for the phenomenon, but this reaction does not alter the striking constancy of the cochlear response under conditions of more moderate stimulation.

Polarity of the electric response. When stimulation of the ear by a tone

is begun abruptly by closing the circuit to the loud speaker, the electrical response begins quite as abruptly as the sound waves recorded by the condenser microphone (fig. 4). The first response is sudden departure from a previously even base-line. This first electric response may be either positive or negative in sign, depending on whether the initial mechanical disturbance is a positive or negative pressure on the ear drum. Positive pressure on the ear drum, produced by a sound wave, causes electrical negativity of the round window relative to the indifferent electrode (cf. fig. 5A). We shall show below that this electrical variation, although correlated in this way with pressure changes, is not generated in the ear drum or round window but in the organ of Corti. During the establishment of a

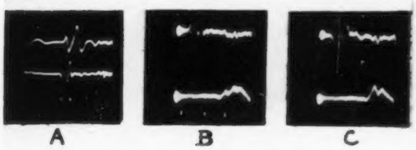


Fig. 5

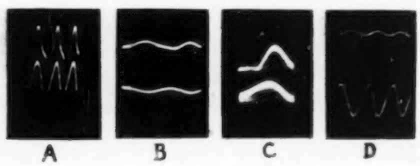


Fig. 6

Fig. 5. (July 26, 1933.) Response to stimulation by single clicks.

A. Upper curve, round window response. Lower curve, condenser microphone. Downward excursion shows positivity of round window or positive pressure on condenser microphone. Dots indicate 1σ interval.

B. Upper curve, round window. Lower, action currents from cochlear nucleus. Dots indicate time intervals of 2.3σ.

C. Same as B, except for reversal of polarity of stimulus.

Fig. 6. (July 26, 1933 and January 13, 1933.) Responses from various regions showing phase relationships.

A. Upper curve, condenser microphone; lower, round window.

B. Condenser microphone and stapes.

C. Condenser microphone and promontory. Frequency for A, B, and C 1200 per second.

D. Internal auditory meatus (upper) and round window (lower). Frequency 1250 (retouched).

steady state at the beginning of a continuous tone there is no significant net displacement of the electrical base-line either positive or negative (fig. 4). Our recording system would be thoroughly competent to disclose such a change, since the records in figure 4 were taken with coupling condensers and grid leaks so large that the potential on the oscillograph required five-eighths of a second to fall to half its initial value after the application of a constant difference of potential to the input. This property of the electric response of the cochlea is in marked contrast to action currents from the auditory pathways which start always in a given direction, depending on the relation of grid and ground leads to the active tracts, irrespective of whether the initial pressure on the ear drum is positive or negative.

The electric response at the oval window is not in phase with the response as recorded from the round window. As a first approximation we may say that when the round window is electrically negative, the oval window is positive, both being measured in relation to the same indifferent electrode. The experiment is performed by recording the stimulating sound wave through the condenser microphone and the cathode ray oscillograph. The movement of the spot on the face of the oscillograph is timed in relation to the electrical stimulating wave by causing the latter to determine the exact moment at which the spot returns to the beginning of its sweep (cf. Garceau and Davis, 1933). The phase relationship which the sound wave bears to the electrical stimulating wave is arbitrary, depending on the time required for transmission of the wave down the tubes from loud speaker to condenser microphone, and also on the grid bias, etc., of the thyratron in the sweep circuit, but it is definite and constant in a given experiment. With the movement of the spot synchronized with the electrical stimulating wave, and with frequency constant, a photograph is next taken of the response from the round window. The electrode is then placed in contact with the stapes and another pair of photographs taken. The response from the stapes is about $\frac{1}{4}$ as large as that from the round window, due probably to the shunting effect of the ossicles, but the relationships of the responses from the two windows to one another may be derived directly from their positions relative to the beginning of the sweep in each case. Four comparisons made in this way at frequencies of 705, 1200, 2000, and 3000 (fig. 6) showed that the response of the oval window led that of the round window by phase angles of 130°, 152°, 150° and 193° respectively. Corresponding measurements are difficult below 700 with our present apparatus on account of prominent harmonics, and above 3000 on account of the small response from the stapes. If we disregard a possible systematic change with frequency, the above measurements give an average of 156°. Similar comparison of the response recorded from the bony promontory between oval and round windows shows that at this point it is practically in phase with the round window, the response of the promontory leading that of the round window by 15° as the average of 4 measurements. The phase relation between the cochlear response at the round window and at the internal auditory meatus was similarly studied in an animal which, owing to a chronic inflammation of the inner ear, gave only the cochlear response and no action currents. Ordinarily the response from the internal meatus contains a confusing component contributed by the action currents of the eighth nerve. The absence of auditory action currents from all parts of the auditory pathways in this animal was amply demonstrated by a fruitless two-hour attempt to detect them, but the cochlear response was normal except for a moderately elevated threshold. The phase relationship was satisfactorily studied at six frequencies

between 355 and 1060. Below 355 harmonics were too prominent and above 1060 the response from the internal meatus was too small. The average of these measurements showed the round window leading the meatus by 88°, the extremes being 76° and 107°, and the deviations not related to frequency.

Latency of cochlear response. Having established the polarity of the response of the round window in relation to pressure in the external canal, we measured the latency of this response with respect to the arrival of a wave at the tympanum. The experiment consisted in delivering to the loud speaker a series of condenser discharges at a frequency of 60 per second from a thyratron stimulator (cf. Garceau and Davis). Such a discharge sets up a brief train of two or three sound waves which is recorded by the condenser microphone (fig. 5A). Following the main waves a series of smaller waves, representing echoes in the tube, may be seen, but these have completely died out before the beginning of the next series. The response to these sound waves is recorded from the round window, the position of the response on the film being determined by synchronizing the movement of the spot with the stimulus as described above. The speed of the spot is determined by measuring a standing wave of known frequency from the beat frequency oscillator. Measurements of such records as are shown in figure 5, and similar measurements made directly on the tube face without photographing, yield an average value of 0.27 σ. The distance from the opening of the small side tube to the tympanic membrane, minus the distance from the corresponding opening on the opposite side of the tube to the face of the condenser microphone, in this experiment was 55 mm. The time required for a sound wave to travel this distance in air at 20° is 0.17 σ. This leaves an unexplained latency of 0.10 σ which we tentatively ascribe to the time required for the wave of pressure to be transmitted through the chain of ossicles and endolymph to the basilar membrane.

In figure 5A it is evident that the frequency of the waves constituting the response of the cochlea differs from that of the sound waves most prominent in the record from the condenser microphone. They are much slower, and may correspond to a disguised low frequency component which can sometimes be detected on careful inspection of the microphone record. But they continue for at least a cycle after the cessation of the stimulus, and probably they represent a resonant frequency dependent on the physical properties of the ear (including the short column of air in the external canal, speculum and side tube). Comparison with figure 4 shows the same period exhibited by the first cycle of response to a tone of 435. Numerous similar observations lead us to the conclusion that the cat's ear tends to respond to the sudden impact of a train of waves of any frequency with a vibration at a period of between 600 and 1000 per second. This same frequency also appears at the beginning and during the decay of the response

to stimulation by a rather strong tone of high frequency. During the decay the frequency progressively decreases during two or three cycles from the frequency of the stimulating tone down to 600 per second (see fig. 4E). At the beginning, while the low frequency waves are diminishing, the waves corresponding to the stimulating tone are gradually increasing. None of this alters the conclusions as to the latency of first response, but raises interesting questions as to the establishment and decay of resonant vibration in the cochlea at different frequencies.

Latency of action currents. This subject will be dealt with more fully in a subsequent paper, but as a preliminary statement we may say, on the basis of stimulation by a sudden click as described above, comparing the response from the cochlear nucleus with that from the round window, that the latency between these two responses in some experiments is as brief as 0.8 σ or 1.2 σ, while in others it is of the order of 3 or 4 σ. The difference probably depends on whether the electrode is in contact with afferent fibers of the eighth nerve or efferent fibers leaving the nucleus. The exact phase of the electric response of the round window in which the action currents are initiated has not yet been determined, and this renders doubtful the exact value to be ascribed to the latency. Figures 5B and C illustrate the type of relationship observed and also the cumulative, monophasic character of the action current response as opposed to the oscillations about zero characteristic of the round window.

Fig. 7. (April 11, 1933.) Upper, condenser microphone response to two tones (1000 and 900), showing beats without difference tone. Lower, round window response, same stimulation, showing beats and difference tone (retouched).

Non-linear distortion in the auditory mechanism. Wegel and Lane (1924) have called attention to the non-linear distortion to be expected in a physical mechanism such as the ear in its transmission of sound waves, and conclude that the sum and difference tones which may be heard when two different frequencies of sound waves are applied simultaneously to the ear are due to this effect rather than to any activity of the central nervous system. To test this conclusion, we generated a tone of 900 per second by the usual stimulating loud speaker and another tone of 1000 per second at the other end of the sound tube by another loud speaker driven by an independent oscillator. The alternate waxing and waning of the resulting electrical waves is shown in figure 7, and in particular the periodic swing of the base-line (the midpoint between successive positive and negative deflections) appears clearly. This slow periodic swing indicates the actual presence of such a frequency, i.e., the difference tone, in the activity of the inner ear, although it is absent from the condenser microphone record. The latter shows only the waxing and waning but with excursions symmetrical about an even base-line.

Response as a function of intensity and frequency. The presence of non-linear distortion in the ear becomes immediately evident when we measure the magnitude of the electrical response of the round window in relation to intensity of stimulation at various frequencies. The relationships are usually far from linear. At low frequencies the situation is complicated by prominent harmonics, generated in part by our stimulating apparatus and in part apparently introduced by the ear itself, but with frequencies of 1000 and above, curves of the type illustrated in figure 8 are obtained.

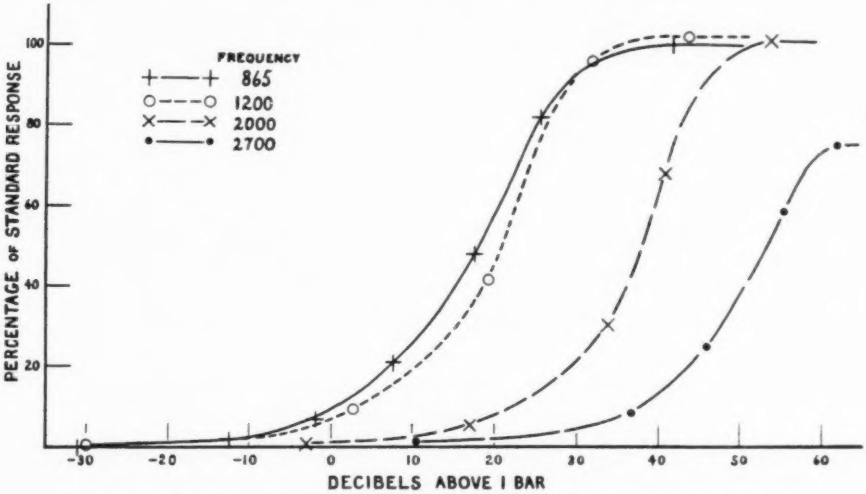


Fig. 8. (July 14, 1933.) Cochlear response as function of intensity of stimulation at frequencies of 865, 1200, 2000, 2700 per second. Intensity of stimulation as measured by condenser-microphone differs somewhat from that actually developed in the external auditory canal. During these measurements the sensitivity of the amplifier was adjusted from time to time to keep the maximal response at frequency 865 always equal to 100 mm. This response is standard of reference, eliminating changes due to any progressive decay. The symbols just above the base-line indicate a just visible threshold response of about ½ mm. Curves corrected for frequency characteristic of amplifier.

Here the responses in microvolts of the round window are plotted against absolute pressure measured by the condenser microphone. The intensity scale is logarithmic, being graduated in decibels (cf. Fletcher, 1929). Zero on the scale corresponds to 1 bar (1 dyne per sq. cm.).[3] Starting with the least visible response on the oscillograph, the typical curve rises first slowly with increase of intensity, then more rapidly, then inflects and finally

[3] All pressures were measured at the condenser microphone. Subsequent tests indicate that they were much less at the ear drum, although the relative values are approximately correct.

reaches a plateau. Our curves differ from one another at different frequencies, in that the threshold value varies, likewise the magnitude of the maximal response, and to a less extent the intensity at which maximum is reached. The shape of the curve is approximately the same in all cases. We have already called attention (p. 316) to the decrease in response resulting from still further increase in intensity above maximal at certain frequencies.

The relation of threshold stimulus to frequency is illustrated in figure 9. This curve corresponds to the familiar audibility curve. It shows a region of greatest sensitivity of the cat's ear to frequencies of 500 to 1000 per second and less sensitivity both above and below. Not only in general

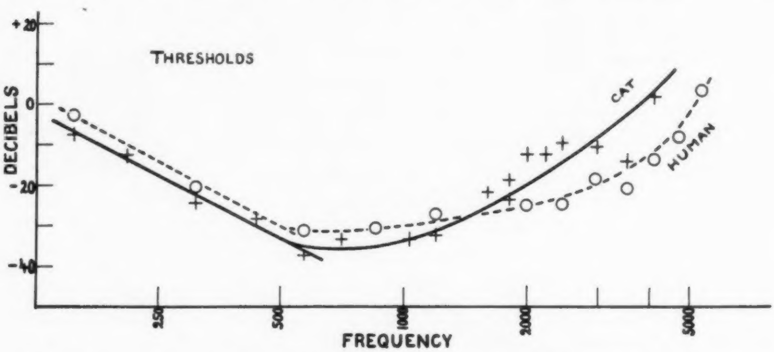


Fig. 9. (Sept. 16, 1933.) Threshold of cochlear response as function of frequency, compared with threshold of human audibility determined with the same stimulating apparatus. Absolute values untrustworthy. The human ear (A. J. D.) had previously been found essentially normal by standard audiometer tests. The low-frequency branch of each curve is drawn straight in conformity with Wegel's (1932) curve. The irregularity of the points in the high frequency range for the cat represent individual characteristics. The smooth curve is a fair approximation to the average.

shape and in the position of the region of greatest sensitivity, but also in absolute values this curve corresponds closely to the human audibility curve. If a cat is mounted as usual on one branch of our sound tube and a human observer applies the other outlet to his ear through a similar speculum, direct comparison of thresholds may be made. As a rule the observer reports thresholds slightly (e.g., about 10 decibels), but consistently, lower than those of the cat. Occasionally the difference is greater than this, particularly for frequencies above 1500 per second, but on several occasions the response from the cat has been clearly observed at intensities too low to be detected by the listener. At high intensities (and medium frequencies) the human observer begins to experience tinnitus and discomfort at very

nearly the same intensity of stimulation as is required to elicit a maximal response from the cat's round window. We do not attach great importance to the exact values of these threshold determinations, since they undoubtedly depend in part on the sensitivity and resolving power of our amplifier, but the trend of the curve and its close correspondence to that of the human are of interest. The absolute values for the human differ from those given by Fletcher.

Figure 10 shows the relation between the magnitude of response to a stimulus of constant energy of varying frequency. This stimulus (40 db)

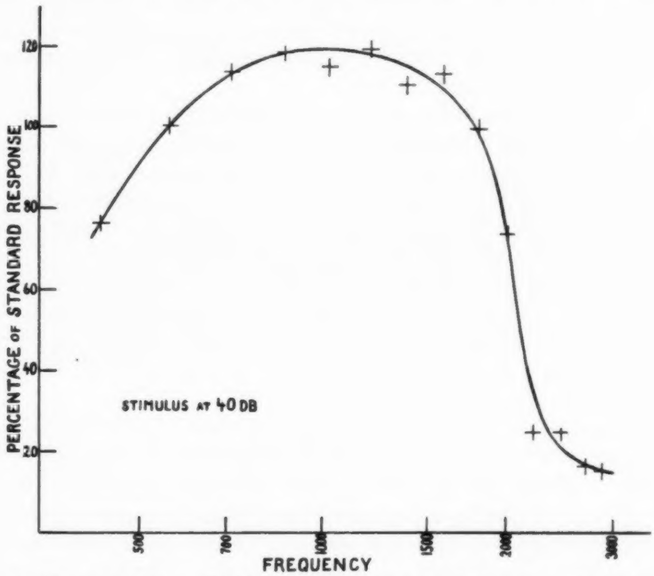


Fig. 10. (July 14, 1933.) Cochlear response to stimulation at 40 db as function of frequency. This curve is obtained from the curves shown in figure 8 and other similar curves at other frequencies corrected for frequency characteristic of amplifier. It is typical of several such experiments. A stimulus of 40 db is sufficient to give rise to tinnitus in the human ear but not to discomfort, over the middle range of frequencies.

is fairly strong; it lies well above threshold for all frequencies studied but at the same time is definitely below maximal. It will be observed that there is a definite maximum in the curve at a frequency range not far above the region of greatest sensitivity of the ear in terms of threshold. The relationships here described between intensity of stimulation, frequency and the magnitude of response serve to explain in part the prominent harmonics which appeared in our earlier records, even though the sound waves used

as stimuli seemed fairly pure. Referring to figures 9 and 10 we see that a fundamental at frequency 432 and intensity of 40 db would yield a response of 77 mm. Assuming a second harmonic of one per cent in the stimulating wave we see that the response to this frequency (865) and intensity (0 db) would be 9.5 mm. which would amount to 12 per cent of the fundamental instead of the original 1 per cent. In addition to this effect, the ear, being a non-linear mechanism, will introduce harmonics even if the stimulus be a pure sine wave (Wegel and Lane, 1926). We have not attempted to differentiate these effects experimentally.

Plotting the maximal response obtainable at a given frequency against frequency we find that the greatest responses are obtained at frequencies between 1000 and 2500. This maximal value is approximately 1 millivolt peak voltage at the round window.

We may note parenthetically that the threshold for detection of action currents in favorable situations in the nervous system of the cat corresponds in order of magnitude with the thresholds obtained for the response of the round window, although as a rule it is 15 or 20 decibels higher. This difference is due in part to masking of the threshold response by spontaneous activity in the nerve centers. The degree of nervous activity as measured by action currents seems to follow the same type of curve as we have described for the response led from the round window, reaching its maximum at very nearly the same intensity. Details of the relation between the action current response and the cochlear response will be reported later by one of us (A. J. D.).

Dependence of the electric response upon the organ of Corti. Significant evidence as to the anatomical structure responsible for the generation of the elctric response was afforded by animals having congenital abnormalities of the ear. It has long been recognized that white cats with blue eyes frequently are deaf, and that the anatomical deficiency in these cases is an absence of the organ of Corti (cf. Alexander, 1900). We tested a white cat having one blue eye and one yellow eye. Preliminary observation over a period of weeks assured us that the animal heard well, being extremely responsive to slight sounds. For the acute experiment the animal was prepared in the usual way with exposure of both round windows and of the mid-brain for study of action currents. The ear on the side corresponding to the yellow eye gave electric responses normal in respect to threshold, magnitude and wave form. Action currents were detected in response to stimulation of this ear from the homolateral inferior colliculus. From the round window on the side of the blue eye, however, no electric disturbance whatever could be detected at a sensitivity of 1000 mm. per millivolt. Neither could action currents be detected in the contralateral inferior colliculus, which is usually extremely sensitive and which was proved responsive to homolateral stimulation. Both inner ears were removed, fixed in

Helley's solution and sectioned for microscopic examination. Figure 11 shows the condition of the organ of Corti on the two sides. It is evident that the hair cells and supporting cells are absent from the unresponsive ear, being replaced by a few connective tissue cells, that the tunnel is absent, that Reisner's membrane is sunken and adherent to the basilar membrane and that the number of nerve fibers in the spiral lamina is markedly reduced. The responsive ear is essentially normal. Further examination showed that the ossicles, the membrane of the oval window, the round window, saccule, utricle and the semi-circular canals were essentially normal in the unresponsive ear. In other words, total absence of electric response is correlated with the deficiencies in the organ of Corti just described. This confirms observations reported by Crowe (1933) on similar animals.

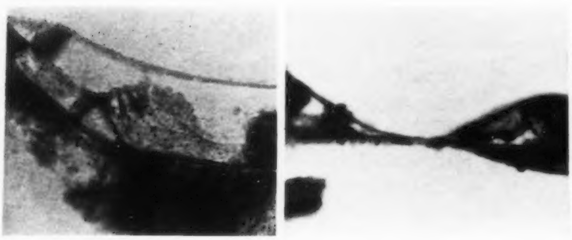


Fig. 11A Fig. 11B Fig. 12

Fig. 11. (May 16, 1933.) Photomicrographs of cochleas from "albino" cat.

A. Responsive ear, high power, showing normal organ of Corti.

B. Unresponsive ear, high power, showing absence of organ of Corti and abnormal position of Reissner's membrane.

Fig. 12. (March 6, 1933.) Section of cochlea of waltzing guinea pig which gave no electrical response from the cochlea. Basilar turn, photographed under high power.

Even more significant is the result of a test upon a waltzing guinea pig. This animal is one of a strain suffering from congenital deficiency of the labyrinthine mechanism, which causes it to run in circles like the familiar waltzing mouse. Rather crude preliminary tests gave no evidence that the animal was sensitive to sound. Examination by our routine procedure gave no evidence of any electric response whatever from the round window, although numerous controls demonstrated the sensitivity of the apparatus. Subsequent histological examination revealed a surprising degree of normality in the cochlea. Not only is basilar membrane present, but likewise the organ of Corti, including tunnel, supporting cells and sensory cells (fig. 12). The latter are so nearly normal in the apical turn that in the original specimen the hairs of the sensory cells may be clearly

distinguished. On the other hand careful examination indicates a moderate but definite degree of degeneration in the sensory cells at least in the basal turns. It is rather more than can reasonably be accounted for on the basis of post-mortem changes, and is more pronounced in the basilar than in the apical turn. Dr. R. Lorente de Nó has examined the original preparations and has kindly allowed us to quote his opinion as confirming the degree of abnormality described above. If we are correct in believing that the response originated in the organ of Corti, this case indicates that absence of the electric response may depend upon a rather slight degree of apparent abnormality in this structure.

Another animal of interest in this connection is one mentioned on page 318 which was suffering from a chronic inflammatory process involving middle and inner ear. The middle ear was full of thick pus and the endothelium was markedly thickened and the bony walls of the bulla eroded. After carefully cleaning the middle ear, the electric response of the round window was tested and found to be normal in respect to magnitude of response, wave form and limits of frequency, although the threshold was slightly elevated. On the other hand, no action currents could be detected at any point tested in the mid-brain of this animal. This situation is unique in our series of over 100 animals except for one possible doubtful case early in the series before our technique was thoroughly established. Histological examination of the ear of this animal, although not as satisfactory as we might wish from the technical point of view, revealed an approximately normal organ of Corti but indicated the probable presence of an inflammatory process in the region of the nerve cells within the bony spiral of the cochlea. It is, therefore, apparent that the action current response may be dissociated from the electric response of the cochlea by a lesion involving the auditory nerve.

Discussion. The various characteristics of the cochlear response which we have described all indicate that it is closely associated with mechanical vibration in the inner ear, most probably in the organ of Corti. Numerous characteristics, such as the threshold curve and the non-linear relationship between stimulus and response, almost certainly depend upon the characteristics of the inner and middle ear as a transmission system for sound waves, and the electric response of the cochlea seems to be ideally suited for study of the modification of this transmission by physiological or pathological conditions (cf. Hughson and Crowe, 1932; also Culler, Finch and Girden, 1933). Two limitations on such procedure should be mentioned, however. In the first place care must be taken not to modify the conditions of electrical conductivity in the neighborhood of the inner ear if conclusions are to be drawn from the magnitude of the electrical response. Secondly, as illustrated by our case of inflammation of the spiral ganglion, if there is injury or abnormality of the nerve itself, the electrical response

of the cochlea will not give a true index of the efficiency of the organ of hearing as a whole. Furthermore it would seem desirable to study the responses at the round window or adjacent regions where they are free from action currents, rather than to record from an unshielded electrode inserted into the 8th nerve. Such an electrode records inevitably a mixture of cochlear response and action currents in uncertain proportions. An electrode on the round window might theoretically alter the mechanical activity of the inner ear, but unless extreme pressure be exerted we have never detected alterations in either amplitude or wave form which we could attribute to this cause.

The characteristics of the electric response of the cochlea indicate very clearly that it is fundamentally different from the action current of nerve or muscle. In the first place it may be either positive or negative with respect to the initial electrical state. Action currents, on the other hand, regularly show a negativity of the active region with respect to an indifferent electrode placed elsewhere on the animal. In spite of certain artefacts of the opposite sign which may either precede or follow the main wave of action current (cf. Bishop and Gilson, 1929) the cumulative effect of a rapid series of action currents is definitely unidirectional. This is illustrated in figure 5 and is in striking contrast with the oscillations about the original zero which characterizes the response of the cochlea.

Secondly, there is no evidence of a refractory period following the development of the electric potential. If it is present it must be of extraordinary brevity since the cochlear response reproduces not only the frequency but also the wave form of the stimulus with fidelity up to at least 8000 per second. Adrian (1931) showed that the ability of the cochlea to reproduce high frequencies is only slightly diminished by cooling. Furthermore the cochlear response is not only immune to fatigue but does not even show the phenomenon of equilibration, i.e., a rapid reduction under high frequency of stimulation to a steady state smaller than the initial response (cf. Gerard, 1932). In nerve and muscle, equilibration appears at frequencies of stimulation much lower than those necessary to disclose, by irregularity of response, the presence of a refractory period (cf. Forbes and Rice, 1929; also Davis and Davis, 1932).

Finally we have found no evidence for an all-or-none relationship in the response of the cochlea. Even at the lowest intensities of stimulation which yield visible responses we have seen nothing suggesting a step-like increase in the magnitude of response. Our methods are probably still too crude to detect the response of a single element, and might for this reason fail to reveal a fundamental quantal character even should it exist. On the other hand our detection of threshold responses at intensities corresponding to the limit of human auditory acuity suggests that we may not be far from such a degree of sensitivity. At all intensities of stimulation

we find the response smoothly graded with respect to both intensity and duration. The waves are as nearly sinusoidal in character as one would expect when allowance is made for their harmonic content. In order to reconstruct such waves from units of definite size and duration, such as the all-or-none concept implies, complicated assumptions as to statistical distribution are necessary. Furthermore, to account for the positive as well as the negative phase of the waves it would be necessary to assume either two types of cell or at least two groups of similar cells inversely oriented with respect to the basilar membrane. No such arrangement is suggested by the anatomical structures; and besides, if we think of any such all-or-none elements we presumably imply that they are either nerve or sensory cells and that each such quantum would represent the process underlying the initiation of a nerve impulse. Two sets of cells inversely oriented would result in a volley of nerve impulses set up during the positive as well as the negative phase of each cycle. The frequency of action currents in the auditory tracts should then be double the frequency of the stimulating sound wave. Actually, with allowance made for harmonic components, we find the frequency of action-current volleys corresponding exactly to the frequency of the stimulating wave up to the frequency at which the action-current response ceases to be synchronized at all (Davis, Derbyshire and Saul, 1933). The entire picture, therefore, is not one of quantal release of energy previously stored in nerve fibers or sensory cells as in the case of action currents, but rather a transformation of energy from the incident sound wave into electricity.

Adequate blood supply to the cochlea is essential for the cochlear response. Following its interruption or on the death of the animal the response falls rapidly to a low level and then either gradually or suddenly disappears completely. This we would interpret as expressing the death of the tissue responsible for the transformation. The residual effect after loss of blood supply cannot be explained by bone conduction of sound to the opposite ear and spread of response back to the electrode, for it disappears on pithing the basilar membrane (cf. p. 316). It represents a persistence of activity at a reduced level for some time after failure of the circulation.

We may undertake to draw certain more specific conclusions as to the origin of the electric response in question. The response arises after the arrival of a sound wave at the tympanum and before the appearance of the corresponding nerve impulse in the acoustic nerve. It may be present when action currents are absent, as with inflammation of the cochlear nerve and immediately after death of the animal, but we have never found action currents in the absence of the cochlear response. On the other hand it arises at or subsequent to the point at which the non-linear distortion of the sound waves is introduced. The total mechanical activity of the

inner ear might be supposed to give rise to it through frictional effects, streaming potentials, or compression of polarized tissues lining its bony cavity, but the relationship between the response at the round and at the oval window definitely rules out the possibility that the potential is generated merely by increased or diminished pressure within the cavity of the inner ear as a whole. We believe that the absence of electrical response from the ears of our white cat without organ of Corti and waltzing guinea pig with abnormal hair cells, and likewise the identity of the function relating response to intensity of stimulation for both cochlear activity and action currents, serve to relate the electrical response specifically to the sensory cells of the organ of Corti. We venture the hypothesis that the electrical potential arises from the sensory cells themselves as a result of mechanical deformation and that it is a continuous function of this distortion whether or not it is linearly proportional to it. From the phase relations between the response of the round and the oval windows, which are in electrical continuity with the scala tympani and the scala vestibuli respectively, we may infer that, however generated, the difference of potential is oriented more or less perpendicular to the basilar membrane. Translated in terms of the hair cells this would imply that the difference of potential is developed between the upper and lower ends of these cells. The magnitude of the cochlear response (as much as 1 millivolt at the round window) is sufficient to make reasonable the further hypothesis that the electrical disturbance is actually the stimulus responsible for the initiation of nerve impulses in the fibers of the auditory nerve. The speed with which the excitatory process occurs, great enough to drive the nerve up to the limit of frequency set by its own refractory period (more evidence on this point will be presented elsewhere), is in favor of this hypothesis. It is conceivable that we have here a clue to the mechanism of activation of nerve fibers by other end-organs which are sensitive to mechanical stimulation. This hypothesis is a development of a suggestion put forward by Hill (1910), but as far as we are aware this is the first phenomenon which might reasonably be interpreted as evidence of an electrical change associated with mechanical deformation of sensory cells. Real or apparent changes in electrical potentials resulting from the deformation of other varieties of cell are familiar. The case of muscle subjected to passive stretch (Einthoven, 1918) probably reveals nothing more than a change of resistance in the circuit, but the electrical change associated with the wave of mechanical deformation described by Osterhout (1931) in *Nitella* is probably fundamentally similar to our phenomenon. The case of retinal potentials, in which a photochemical rather than a mechanical process is clearly involved, does not fall in this class, although it is of interest that all of these cases, like the cochlear response, fail to show any all-or-none relationship. We believe that the sensory cells and nerve fibers of the ear are

analogous to the cutaneous tactile receptors studied by Adrian, Cattell and Hoagland (1931). These investigators were able to obtain frequencies up to 400 per second in the response of a single sensory unit to rapid mechanical stimulation. In terms of Adrian's characterization of end-organs we should say that the auditory receptor mechanism, like this tactile mechanism, is one characterized by rapid adaptation.

Continuing this analogy along the lines pointed out by Adrian (1932) we suggest that, as in the case of these cutaneous tactile receptors in which increase in intensity of stimulation is reflected in the activation of greater numbers of neural elements and not in increase in the frequency of discharge of individual elements, the frequency of discharge is determined by the frequency of the stimulating sound waves so far as the nerve fiber is able to keep pace. If differences of frequency of auditory nerve impulses have a psychological correlate it is probably to be found in some quality of sensation other than intensity. On the other hand, our failure to detect synchronized action currents in response to stimulation at frequencies above 2500 (Davis, Saul and Lurie, 1933) seems to point inescapably to a place theory for the discrimination between tones of high pitch. Wever (1933) has ably summarized the status of the leading rival theories of hearing and pointed out difficulties in the way of each. At present it seems possible that the mechanism responsible for discrimination between two tones of low pitch may actually be different from that which discriminates among tones in the high range, but we do not undertake to reach a decision on this point until further evidence has been accumulated.

We wish to express our thanks to Mr. T. Butler and particularly to Dr. C. L. Prosser for assistance in the performance of many of these experiments.

SUMMARY

When sound waves fall upon the ear of an anesthetized cat, an electric response, distinct from nervous action currents, is generated in the cochlea. When transformed again into sound it reproduces with great fidelity spoken words and pure tones.

The response measured at the round window of the inner ear may amount to one millivolt.

Positive pressure on the ear drum causes a fall and negative pressure a rise in the electrical potential of the round window. The opposite relation holds for the oval window (p. 317).

The response is only slightly depressed by increasing anesthesia until the circulation fails. It then falls rapidly to from five to twenty per cent of its original strength and more slowly to extinction in one-half to several hours (fig. 2).

The response is immediately abolished by pithing the basilar membrane.

The latency of the first electrical response following the arrival of a sound wave at the ear drum is about 0.1 σ.

The latency of the action currents in the eighth nerve is of the order of 1 σ with respect to the cochlear response (p. 319).

The electric response follows the frequency of the stimulating tone up to at least 8000 per second. The response to the sudden impact of a train of sound waves at any frequency is a rapidly decrementing series of electrical oscillations of a frequency between 600 and 1000 per second. This "on effect" merges into the response at the frequency of the stimulating tone. After cessation of stimulation with a strong tone above 1000 per second a similar series of decrementing oscillations appears (figs. 4 and 5).

The cathode ray oscillograph shows that the wave form of the stimulus is reproduced accurately except for non-linear distortion by the ear. This distortion includes the introduction of higher harmonics and relative accentuation of those already present.

The cochlear response reveals the presence of a "difference tone" when the ear is stimulated by two tones simultaneously. This is further evidence of non-linear distortion (fig. 7).

The intensity of the cochlear response is not a linear function of the strength of the stimulus as measured by a condenser microphone. The curve relating it to the logarithm of the stimulus is sigmoid in shape, reaching its maximum at approximately the intensity which causes discomfort to the human ear (fig. 8).

The curve relating threshold stimulus to frequency closely resembles the human audibility curve, both in absolute values and in the range of maximum sensitivity (fig. 9).

The cochlear response was present but auditory action currents were absent in an animal showing inflammation of the nerves within the bony spiral of the cochlea.

The cochlear response and also action currents were absent from a cat showing congenital absence of the organ of Corti and from a waltzing guinea pig showing no gross anatomical deficiency but merely partial degeneration of the sensory hair cells (p. 324).

The utility of the cochlear response as a tool for the analysis of auditory function is pointed out. The theoretical implications of the data are discussed. The cochlear response is interpreted as arising in the sensory cells of the organ of Corti as a result of mechanical deformation.

REFERENCES

Adrian, E. D. 1931. Journ. Physiol., **71**, Proc., xxviii.
1932. The mechanism of nervous action. Philadelphia and London.

Adrian, E. D., D. W. Bronk and G. Phillips. 1931. Journ. Physiol., **73**. 2p.

Adrian, E. D., McK. Cattell and H. Hoagland. 1931. Journ. Physiol., **72**, 377.

Alexander, G. 1900. Arch. f. Ohrenheilk., **50**, 159.
Bast, T. H., R. Noer, R. West, O. L. Backus, M. Krasno and J. A. E. Eyster. 1933. Proc. Soc. Exp. Biol. and Med., **30**, 638.
Bishop, G. H. and A. S. Gilson, Jr. 1929. This Journal, **79**, 135.
Crowe, S. J. 1933. Discussion reported in Trans. Amer. Otol. Soc., in press.
Culler, E., G. Finch and E. S. Girden. 1933. Science, **78**, 269.
Davis, H., A. J. Derbyshire and L. J. Saul. 1933. This Journal, **105**, 27.
Davis, H. and P. A. Davis. 1932. This Journal, **101**, 339.
Davis, H. and L. J. Saul. 1933. Amer. Journ. Psychol., **45**, 358.
Davis, H., L. J. Saul and M. H. Lurie. 1933. Trans. Amer. Otol. Soc. In press.
Einthoven, W. 1918. Arch. Néerland. de Physiol., **2**, 489.
Fletcher, H. 1929. Speech and hearing. New York.
Forbes, A., R. H. Miller and J. O'Connor. 1927. This Journal, **80**, 363.
Forbes, A. and L. H. Rice. 1929. This Journal, **90**, 119.
Garceau, L. and H. Davis. 1933. This Journal, in press.
Gerard, R. W. 1932. Physiol. Rev., **12**, 469.
Hill, A. V. 1910. This Journal, **40**, 190.
Hughson, W. and S. J. Crowe. 1932. Ann. Otol., Rhinol. and Laryngol., **41**, 332.
Osterhout, W. J. V. 1931. Journ. Gen. Physiol., **14**, 473.
Saul, L. J. and H. Davis. 1932. Arch. Neurol. and Psychiat., **28**, 1104.
Wegel, R. L. and C. E. Lane. 1924. Phys. Rev., **23**, 266.
Wegel, R. L. 1932. Ann. Otol., Rhinol. and Laryngol., **41**, 740.
Wever, E. G. 1933. Physiol. Rev., **13**, 400.
Wever, E. G. and C. W. Bray. 1930a. Science, **71**, 215.
1930b. Proc. Nat. Acad. Sci., **106**, 344.
1930c. Journ. Exper. Psychol., **13**, 5.

SEASONAL AND TEMPERATURE FACTORS AND THEIR DETERMINATION IN PIGEONS OF PERCENTAGE METABOLISM CHANGE PER DEGREE OF TEMPERATURE CHANGE

OSCAR RIDDLE, GUINEVERE C. SMITH AND FRANCIS G. BENEDICT

From the Station for Experimental Evolution, Carnegie Institution of Washington, Cold Spring Harbor, N. Y., and the Nutrition Laboratory, Carnegie Institution of Washington, Boston, Mass.

Received for publication September 26, 1933

In the case of a few animals it has been observed that the basal metabolism is more or less influenced by season, but in no animal has a thoroughgoing study of the seasonal factor been made. We here record the result of an effort to obtain a satisfactory measure of the influence of season on the metabolism of one race of common pigeons. We find this influence to be of the order of 10 to 15 per cent in birds given considerable protection from cold during winter and spring, and kept behind walls of ordinary glass at those two seasons only. This result is especially significant because it was obtained on animals offered an unvarying diet of dry grain-mixture at all seasons of the year; since we can note no evidence that any particular grain of this mixture is especially preferred or shunned at any season the fluctuations of metabolism observed here are probably not referable to seasonal changes in the dietary.

In metabolism work it is recognized that the heat produced by many test animals varies with the environmental temperature at which the measurements are made; and, in connection with such studies the statement is commonly made that a degree of change in the external temperature—at a particular region of the thermometric scale—corresponds to a certain percentage change in the metabolism. So far as we are aware the *effects of season* upon this *percentage change* of the metabolism with each degree of change in the environmental temperature have nowhere been considered. In this study (1) we find that something associated with season essentially determines whether the metabolism is modified much or little by each unit of change in environmental temperature between 15° and 30°C.

TECHNIQUE AND MATERIAL. All measurements were made with the multiple chamber apparatus (2), at night, in fully darkened chambers, and with all the technique earlier found (3) necessary to the accurate measurement of basal heat production in these animals. We especially note that these birds spent their 24-hour fasting period, just preliminary to measure-

ment, in a large constant temperature cage maintained at that particular temperature at which their metabolism was to be measured. When placed in the respiration chamber for measurement these birds were therefore in an atmosphere of the same temperature (15°, 20°, or 30°C.) as that in which they had lived during the preceding 24 hours.

For this study we chose a single well-marked race of common pigeons—the tipplers. In this material the possibility of marked genetic difference due to contamination with another race is excluded. Only a single stage in the life cycle of this animal was utilized, namely, that of the mature relatively young animal (6–28 months old) mated and in active reproduction. The males and females, usually brothers and sisters, were taken for measurement at the same time, and the values for the two sexes are treated separately. The average age is similar for the various tabulated groups, with the exception that most of the groups measured at 30° are about 2 months younger than other groups; correction for the age factor there would slightly diminish the 30° values as tabulated. No birds were measured while incubating eggs nor while feeding young; but in this early-maturing and actively reproducing race it was rarely possible (except in autumn) to obtain female birds not approaching ovulation and whose oviducts were not at least partly hypertrophied (they increase 1000 per cent in weight) and motile. Whatever heat is produced by this temporary growth and motility (both entirely absent during the prolonged periods of incubation and feeding of young), together with that arising from other important temporary conditions (probably including thyroid hyperplasia) known to accompany ovulation in these birds (4), (5), is necessarily but wrongly included in the "basal" values recorded for these females. This inclusion of temporarily high values for many females would appear to account fully for the observed apparent difference in heat production of the two sexes.

Measurements, 429 in number, were made during all seasons at three environmental temperatures, 15°, 20°, and 30°C. The data were obtained during no single year, but during 3 successive years. Most of the birds used were measured repeatedly—at two or three temperatures during a season, and in many cases at two or more seasons. Measurements made during December, January and February are here called "winter" measurements; those of June, July and August form the "summer" groups. It is important to note that from near the end of November to the end of April all mature and mated birds of our colony are kept behind glass or other walls which are removed during other months of the year. Thus these birds are subjected to ultra violet rays during summer and autumn but not during winter and two-thirds of spring. Moreover some artificial heat is supplied during winter and (early) spring when the presence of glass walls further assures the lower cooling power of *still* air.

Consideration of data. *Seasonal changes of the metabolism.* It will be observed (table 1) that at an environmental temperature of 20°C. the values obtained (for both males and females) during autumn are definitely higher than those found at other seasons; summer values rank second (fourth in females when corrected for the age factor), with winter apparently third and spring fourth and lowest. Measured at 15°, however, autumn values certainly do not show the same superiority over summer values; our data (for both sexes) on this point indicate that birds measured at 15° in summer have a slightly higher metabolism than birds in autumn measured at this temperature. Our 30° measurements indicate no difference in summer and autumn values for females, and perhaps only a questionably higher autumn value for males. Here again, as in 15° measurements, spring and winter values are lowest and practically indistinguishable from each other.

Measurements made at 15° and 20° reveal marked seasonal differences (in both sexes); those made at 30° seem to minimize such seasonal differences in females and still to exhibit them in males. The aberrant value for the female in these 30° measurements is the unexpectedly low metabolism in autumn; this may have the following explanation: First, ovulation rate is lowest in autumn and changes accompanying ovulation will then least often of all seasons temporarily raise the metabolism above its true basal value. Second, the birds in autumn—accustomed then to air of greatest cooling power—will be most affected (insulted?) by placement in a temperature of 30°; and, since the ovary is highly sensitive to all adverse influences it will begin to involute soon after subjection to 30° (this is 24 hours before the metabolism measurement begins) and diminish its production of oestrin—on which the growth and motility of the oviduct and still other changes at ovulation depend. It is practically certain that these changes occur, and their occurrence would account for an autumn value such as was obtained.

In this material we should be able to associate seasonal changes in heat production with seasonal changes in endocrine (thyroid and gonad) activity and with seasonal changes in amount of ultra violet light absorbed by these animals. Riddle and Fisher (6) measured the seasonal changes of thyroid size in several races of doves and pigeons (tipplers not included) and found smallest size in summer and largest size—with histological evidence of greatest activity—in autumn (or winter). Haecker (7) later obtained similar results in the (non-captive) European crow. It is therefore probable that such seasonal changes in the thyroids occur widely and almost certainly in the tippler pigeons used in the present study. Riddle (8) also showed that in the birds of this colony ovulation rate is greatly reduced and the size of the gonads measurably reduced during the autumn (less reduced in winter) in doves and pigeons generally; also that ovula-

TABLE 1

Summary of metabolism measurements made during all seasons at 15°, 20° and 30°C. on healthy adult common pigeons of the same race (tipplers)

TEMPERATURE USED	SEASON	MALES					FEMALES					PERCENTAGE EXCESS METABOLISM IN FEMALES
		Number of tests	Age	Body weight	Calories per kilo per 24 hours	Average temperature	Number of tests	Age	Body weight	Calories per kilo per 24 hours	Average temperature	
			mos.	*gms.*		*°C.*		*mos.*	*gms.*		*°C.*	
30°C.	Spring....	18	11.4	275	90.5	30.07	18	12.1	264	104.3	30.09	14.5
	Summer..	11	11.4	255	99.7	30.11	17	11.2	247	105.7	30.10	6.0
	Autumn..	20	11.7	264	104.7	30.06	20	11.1	265	105.7	30.06	1.0
	Winter...	14	12.4	284	93.0	30.12	14	12.6	276	101.2	30.03	8.8
Yearly mean...		63	11.7	270	97.0 627*	30.09	69	11.7	263	104.2 667*	30.07	7.5 6.4
20°C.	Spring....	21	14.1	276	97.2	19.98	27	13.8	254	106.8	19.96	9.9
	Summer..	19	13.5	266	104.5	19.98	19	12.2	259	107.6	20.00	3.0
	Autumn..	21	14.8	269	112.5	20.01	21	14.4	258	115.0	20.13	2.2
	Winter...	21	13.4	279	101.1	19.95	23	14.2	259	109.2	19.96	8.0
Yearly mean...		82	14.0	273	103.8 674*	19.98	90	13.6	257	109.6 697*	20.01	5.6 3.5
15°C.	Spring....	14	13.0	281	106.2	15.04	15	13.9	271	117.6	15.04	10.7
	Summer..	14	12.7	263	124.4	15.09	18	13.7	251	129.5	15.04	4.1
	Autumn..	12	14.2	256	120.3	15.14	15	12.5	253	124.3	15.05	3.3
	Winter...	18	14.4	278	112.9	15.04	19	13.2	276	115.3	14.99	2.1
Yearly mean...		58	13.6	269	116.0 748*	15.08	67	13.3	263	121.7 778*	15.03	4.9 4.1

* Calories per square meter per 24 hours ($S = 10 \times W^{\frac{2}{3}}$; S = square centimeters, W = body weight in grams).

tion rate and gonad-size reach their maximum in spring. Seasonal differences in activity of gonads were slight and unimportant in the male birds used in the present study. Thyroid states would lead one to expect highest metabolism values in autumn and winter, and lowest in summer; but highest values are actually obtained in autumn and summer. It is evident that the seasonal fluctuations observed in the present series of measurements can not be interpreted solely on the basis of seasonal changes in thyroids and gonads.

The birds studied here were exposed to ultra violet light during only summer and autumn (early May to near end of November), and these are the seasons of high metabolism; glass or other walls practically excluded ultra violet rays from these birds during winter and spring, and these are the seasons of lowest basal heat production. We are unable to state whether quantity of light other than ultra violet is of significance in our results; but we note that the short days of winter necessarily restrict the daily period of exercise (and food intake?), in these animals, and the removal of glass sides of the colony houses served to admit slightly more of the visible rays in addition to providing free and moving air. At all temperatures used for measurement the metabolism values for summer are higher than those for winter and spring, and—since thyroid activity is probably at its minimum in summer—we are able to interpret this particular difference only as a response to ultra violet light or to generally increased radiation and (perhaps) to open air. Both summer and autumn birds are much exposed (a narrow roof is present) to ultra violet, but temperature and thyroid activity (in races hitherto measured) are quite unequal in these two seasons and we interpret the higher metabolism in autumn (at 20° and 30°) as a response to increased thyroid activity during this season. Only measurements made at 15° show a different or opposite relationship of summer and autumn metabolism values, and this exceptional result very probably reflects differential stimulating effects of this very low temperature. During autumn the birds have been living in air of great cooling power—their mechanisms of heat production and heat loss have been adjusted to this high degree of cooling power—and when placed in the metabolism chamber at 15°, this temperature has relatively *little* stimulating effect; during summer the birds have been living at a high external temperature—their mechanisms of heat production and heat loss have been adjusted to this low degree of cooling power—and when placed in the metabolism chamber at 15° a relatively great *stimulating* effect of this temperature is obtained.

Inspection of the data further suggests that relatively larger *depressant* effects are obtained on autumn and winter birds (than on spring and summer birds) when these are measured at 30°—a temperature far higher than that to which the birds are then currently accustomed. Again, though

winter birds probably had more active thyroids and were more recently exposed to ultra violet light than those of spring, the latter birds are indicated by 30° measurements as having the higher metabolism. The 20° measurements show the opposite and expected relationship and thus provide some evidence that this temperature, rather than the "critical" temperature (approximately 30°) is the more acceptable one at which to measure metabolism in these animals.

TABLE 2

Showing in tippler pigeons the influence of season on the percentage increase or decrease of metabolism per degree of temperature change (from 20°C.)

BASIS OF COMPARISON (20° VALUES)				THINGS COMPARED (30° AND 15° VALUES)				PERCENTAGE METABOLISM CHANGE (+ OR −) PER DEGREE TEMPERATURE CHANGE (FROM 20° VALUE)
Season	Chamber temperature (°C.)	Calories per kilo per 24 hours (mean of ♂ and ♀ values)	Number of tests	Season	Chamber temperature (°C.)	Calories per kilo per 24 hours (mean of ♂ and ♀ values)	Number of tests	
Summer	20°	105.5	38	Spring	30	97.9	36	−0.72
				Summer	30	102.7	28	−0.28
				Autumn	30	105.2	40	−0.03
				Winter	30	97.1	28	−0.79
				Spring	15	111.9	29	+1.21
				Summer	15	127.0	32	+4.11
				Autumn	15	122.2	27	+3.23
				Winter	15	114.1	37	+1.63
Autumn	20°	113.7	42	Spring	30	97.9	36	−1.39
				Summer	30	102.7	28	−0.97
				Autumn	30	105.2	40	−0.75
				Winter	30	97.1	28	−1.45
				Spring	15	111.9	29	−0.32
				Summer	15	127.0	32	+2.32
				Autumn	15	122.2	27	+1.50
				Winter	15	114.1	37	+0.06

Influence of season in determining percentage metabolism change per degree of temperature change. The condensations and calculations of data given in table 2 provide a quantitative expression of the rôle of season in deciding how much the metabolism of summer and autumn birds has been changed by raising the temperature from 20° to 30°, or by lowering it from 20° to 15°. Measurements made at 20° on summer and autumn birds only are thus utilized because these two seasons represent the extremes of temperature (cooling power) to which our birds were currently accustomed,

and because light (radiation) conditions were not experimentally changed by us during these two seasons.

It is readily seen that the rôle of season—i.e., the changes effected in the organism by things associated with the (astronomical) progression of the seasons—is of first importance. Whether one measures a summer bird at 20° or at 30° is of very little consequence since the two metabolism values are found to differ by only 2.9 per cent (0.28 per cent per degree); but a lowering of the environmental temperature of a summer bird by only 5° (from the 20° base) increases its metabolism by 20 per cent (4.1 per cent per degree). If instead of a summer bird one uses an autumn bird for these same comparisons one obtains a quite different result; then the 30° measurement decreases the metabolism by 0.75 per cent per degree, while at 15° it is increased by only 1.5 per cent per degree. Seasonal groups that are least affected by a rise in temperature are those affected most by lowering the temperature, and *vice versa.* Again, a spring or a winter animal (kept under glass) measured at 15° has the same (or insignificantly less) metabolism as that of an autumn bird measured at 20°. Also, an autumn bird measured at 30° has the *same* metabolism as a summer bird at 20°.

These results demonstrate hitherto unknown precautions and limitations that attach to a statement that a degree of change in temperature corresponds to a certain percentage of the metabolism. In work with our animals certainly, and with many other animals probably, we conclude that it is not alone the environmental temperature at which measurement is made, but the *organism* in its changeable physiological stadia—attained in association with season—that mainly decides whether and to what extent the metabolism will change with change of temperature. Further, these results make it evident that the so-called zone of thermal neutrality, or "critical" temperature, *can not be the same at all seasons for one and the same tippler pigeon.* Indeed, the present demonstration of metabolism change in the changing organism adds unsuspected difficulties to the problem of selecting a temperature—or a zone of thermal neutrality—at which a prolonged series of metabolism measurements should be made on any animal.

Other data bearing on present problems. Riddle and others (unpublished) have obtained some data on the weight of the adult plumage at different seasons in several races of doves and pigeons. The data obtained for tippler pigeons indicate that the males here have relatively heavier plumage (12 grams) than the females ($10\frac{1}{2}$ grams). This is significant in indicating a greater protection against heat loss in the males of this race—probably an important matter—and it also suggests that the inclusion of the plumage weight (non-metabolizing tissue) in the body weight (on which all our calculations of heat production are based) has very slightly

and differentially affected the metabolism values as calculated for the two sexes. This point will be investigated further. In order to eliminate all temporary changes connected with ovulation, as sources of sex and seasonal difference a study of this race in early adolescence at all seasons is in progress. In that study the light factor is also being left unchanged by us throughout the year.

PREVIOUS WORK AND COMMENT. The available discordant data doubtfully show an effect of season on the basal metabolism of man; but perhaps this doubt—and a relatively slight effect of season—mainly serve to emphasize the fact that man has unusual means of making and modifying his own intimate environment. Such an effect was reported particularly by Gessler (9). Gustafson and Benedict (10) with a series of college students noted a tendency for the metabolism to be at a lower level during the winter than in the spring and summer. On the related subject—the influence of a tropical climate—de Almeida (11) and Sundstroem (12) found, contrary to others, that its effect is to decrease the metabolism of man. The seasonal effect, with higher winter and lower summer values, has been reported for swine by Capstick and Wood (13). Rats transferred to air of low cooling power were found by Sundstroem (14) to acquire lower metabolic rates. Kayser (15) reported a similar result obtained on three pigeons; but in order to maintain these birds in air having a temperature of 28°C. they were apparently confined for 31 days in an "incubator," and it seems uncertain whether the result is ascribable to the high temperature, to prolonged close confinement or to restriction of radiation.

The effect of season on the size and activity of the thyroid glands and gonads of both birds and mammals is now fairly well recognized and the earlier literature can be obtained from studies already cited (6), (8). Lazowsky (16) observed that the testes of white mice placed at a temperature of 32° to 35°C. respond differently at different seasons; in autumn and winter they show pronounced degeneration of the seminal epithelium, but in spring and summer they suffer little or no change. Verzar and v. Arvay (17) maintained that the female sex hormone (theelin or menformon) injected into rats, or secreted by its own ovary at estrus, increases the basal metabolism of the rat by fully 10 per cent through a marked increase in the growth and motility of the uterus. v. Arvay (18) reports a 156 per cent increase in O_2-consumption in excised uterine tissue of rodents previously injected with the sex hormone of the ovary. Burge and Leichsenring (19) found least blood catalase in summer, most in winter, and consider catalase content and oxidation rate as directly related values.

Contradictory results on the effect of ultra violet light on the basal metabolism in man and animals have been reviewed by Laurens (20). That radiation markedly affects gonad, thyroid and parathyroid states in birds is unquestionable, and some such effects have been observed—

though not uniformly—in mammals. Extending daylight periods by means of electric light has been shown by Rowan (21) and Bissonnette (22) to activate the gonads of various wild birds. Whetham (23) concludes that the supply of light is an important factor in the seasonal distribution of egg production in fowls. Ultra violet light in winter increases egg production and calcium metabolism in fowls (24); its absence is markedly goitrogenic (25) and also adverse to normal parathyroid development in young fowls (26). Grant and Gates (27) found that in rabbits Hg-lamp irradiation causes hyperplasia of the parathyroids, with perhaps a delay or avoidance of a winter decrease in size of the hypophysis and effects on thyroids and adrenals.

In general we interpret previous work as having established a high probability that prolonged effects of ultra violet light (along with free moving air), and the thyroid hypertrophy which accompanies cold weather, both lead to increased basal heat production in some species; our present results confirm both of these associations in a race of pigeons. Since these two agencies affect the metabolism in this animal, as prepared and studied by us, it is clear that the autumn metabolism should be the highest and that of spring probably the lowest. Since measurements made at 20° do, and those made at 15° and 30° do not, show this expected seasonal relationship the present results supply evidence that a lower temperature (here 20°) than the critical temperature (approximately 30°) is preferable for heat production measurements on these animals. The utilization of any particular high temperature—however well it may drive the rate of heat production to its irreducible minimum—may be held in suspicion if it also masks or reverses the fairly well established relationships of thyroid activity to the basal metabolism.

SUMMARY AND CONCLUSIONS

On healthy adult tippler pigeons in active reproduction 429 measurements were made under basal conditions at all seasons at 15°, 20° and 30°C. From November to early May the animals used were supplied considerable protection from cold by heating and by glass walls which excluded ultra violet light; they were exposed to air of greatest cooling power in autumn.

The measurements made at the three temperatures are not in good agreement as to the relative order of the metabolism at the four seasons; but the data thus obtained and other considerations lead to the conclusion that the 20° measurements present a truer picture of seasonal differences in metabolism than do either the 15° measurements or those made at the so-called critical temperature—approximately 30°C. The full extent of the seasonal difference, when measured at 20°, is 15 per cent in males and 8 per cent in females—with lowest metabolism values in spring and highest in autumn.

The females measured here gave values somewhat higher than true basals

due to marked temporary changes associated with the ovulation stage of the reproductive cycle. The sex differences as tabulated do not represent the true value of the sex factor.

All measurements indicate a higher metabolism in summer than in spring and this difference is apparently due to radiation effects—particularly to ultra violet—rather than to temperature, gonad or thyroid differences at these two seasons.

It is found that the percentage metabolism change per degree of temperature change is a function of season, and of the particular range of the thermometric scale which is used in measurement. In this race one, or even ten, degrees of change in temperature may yield zero per cent or one degree may yield a 4 per cent change in the metabolism according to the season and the temperature range chosen for measurement.

REFERENCES

(1) Riddle, O., G. C. Smith and F. G. Benedict. This Journal, 1932, **101**, 88.
(2) Benedict, F. G. Journ. Nutr., 1930, **3**, 161.
(3) Benedict, F. G. and O. Riddle. Journ. Nutr., 1929, **1**, 475, 497.
(4) Riddle, O. Proc. Amer. Phil. Soc., 1927, **67**, 497.
(5) Riddle, O. Endocrinol., 1929, **13**, 311.
(6) Riddle, O. and W. S. Fisher. This Journal, **72**, 464.
(7) Haecker, V. Schweizer med. Wochenschr., 1926, **15**, 337.
(8) Riddle, O. This Journal, 1925, **73**, 5.
(9) Gessler, H. Pflüger's Arch., 1925, **207**, 370.
(10) Gustafson, F. L. and F. G. Benedict. This Journal, 1928, **86**, 43.
(11) de Almeida, A. O. Journ. Physiol. et Path. Gen., 1922, **22**, 12.
(12) Sundstroem, E. S. Univ. Calif. Publ. Physiol., 1926, **6**, 1.
(13) Capstick, J. W. and T. B. Wood. Journ. Agric. Sci., 1922, **12**, 257.
(14) Sundstroem, E. S. Univ. Calif. Publ. Physiol., 1930, **7**, 103.
(15) Kayser, C. Compt. Rend. Soc. de Biol , 1929, **101**, 708
(16) Lazowsky, J. M. Virchow's Arch. Path. Anat. u. Physiol., 1931, **280**, 311.
(17) Verzár, F. and A. v. Arvay. Biochem. Zeitschr., 1931, **240**, 28.
(18) v. Arvay, A. Endokrinol., 1933, **13**, 9.
(19) Burge, W. E. and J. M. Leichsenring. Journ. Lab. Clin. Med., 1922, **8**, 33.
(20) Laurens, H. Physiol. Rev., 1928, **8**, 1.
(21) Rowan, W. Proc. Boston Soc. Nat. Hist., 1929, **19**, 151.
(22) Bissonnette, T. H. Physiol. Zool., 1931, **4**, 542.
(23) Whetham, E. O. Journ. Agric. Sci., 1933, **23**, 383.
(24) Hart, E. B., H. Steenbock, S. Lepkovsky and S. W. F. Kletzien. Journ. Biol. Chem., 1925, **65**, 579.
(25) Turner, K. B. Proc. Soc. Exper. Biol. and Med., 1930, **28**, 204.
(26) Nonidez, J. F. and H. D. Goodale. Amer. Journ. Anat., 1927, **38**, 319.
(27) Grant, J. H. B. and F. L. Gates. Journ. Gen. Physiol., 1924, **6**, 635.

STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

XXXIII. Body Size Changes in Doves and Pigeons Incident to Stages of the Reproductive Cycle

OSCAR RIDDLE and PELA FAY BRAUCHER

From the Station for Experimental Evolution, Carnegie Institution of Washington, Cold Spring Harbor, L. I., N. Y.

Received for publication October 3, 1933

Rapid and marked changes in the behavior, endocrine-status and basal metabolism have been noted in earlier papers of this series to characterize the reproductive cycle of doves and pigeons. Few species could better serve to show that in all studies on animals it is not merely with an organism, but with a *changing* organism, that one is obliged to work. In these birds it is easy to follow several pronounced cyclic changes which clearly reveal regulation by one or another part of the endocrine system. The present paper records measurements of cyclic changes in body size—one aspect of which may be true growth—, and supplies data on food consumption during certain stages of the cycle.

Methods. Adult breeding pairs of common pigeons (various races) and of ring doves were left in individual full-sized cages (nearly 2 cubic meters) and permitted to complete one or more reproductive cycles under measurement. The measurement involved daily weighings of food consumed, and the weighing of the two parent birds at two or three day intervals; crop-contents, when present, were carefully estimated and deducted from the total weight of the birds. The various pairs were thus measured over a period of six or seven weeks. In order that difference in exposure to cold at various periods of the cycle might not differentially influence the average values for food consumption at the various stages of the cycle these tests were made during spring and summer. Further, at the beginning of the test it was arranged that the several pairs should be in different stages of the reproductive cycle—some incubating eggs, others feeding young, still others in the "resting" or copulatory stage. In most cases the stage or stages first measured was again measured before the conclusion of the study.

Results of measurements. *Changes in body weight.* The average changes in body weight are graphically shown in figure 1 for 28 common pigeons and 30 ring doves. In both of these species the body weight of males and females alike markedly increases while incubating eggs and at-

tains its highest value at the end of that period. Only one of the 58 individual birds failed to show a greater body weight at this period than at any other period of the entire reproductive cycle. Weights of females were corrected for eggs contained in oviducts, but correction was not made for the temporary enlargement of the oviduct itself. The "normal" weight of these groups of birds may be calculated with fair accuracy by taking an average of the values found at the "resting" stage and at the

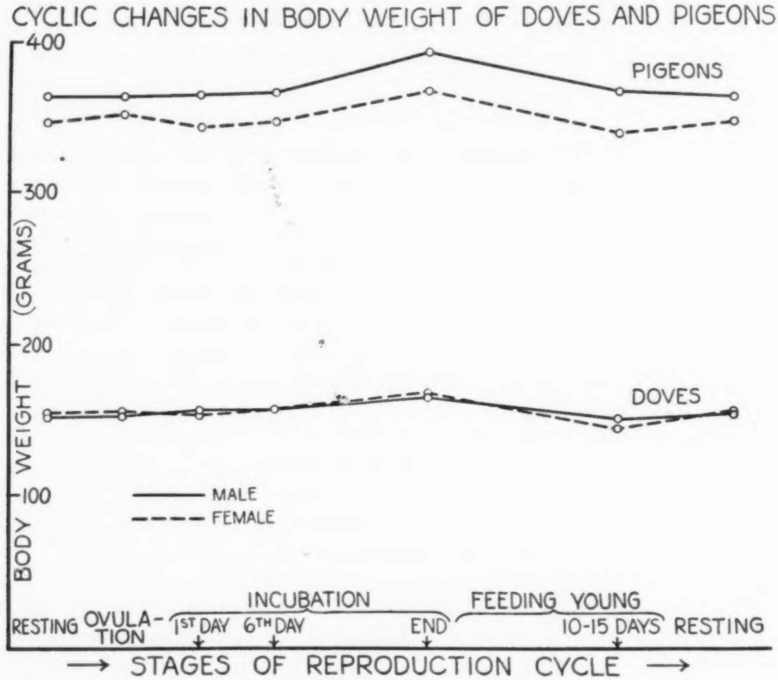


Fig. 1

first day of incubation. The value at the "ovulation" stage can also be included in this average in the case of males, but female weights are then 2 or 3 per cent too high because of the marked temporary growth of the oviduct which occurs coincident with that period. With the "normal" weight thus determined it is found that at the end of incubation this weight was increased in common pigeons by 8 per cent (♂♂) and 7 per cent (♀♀); in ring doves, by 7.5 per cent (♂♂) and 8.5 per cent (♀♀). For the 58 birds this average is 7.7 per cent. Elsewhere (1) the body weights of 63 ring doves, after fasting for 30 hours, were shown to be 7.2

per cent higher when measured within the last 4 days of incubation than when measured in "resting" and "ovulation" stages.

Another period of marked change in the body weight of these two kinds of birds occurs—during the "feeding of young" stage—immediately after the maximum weight is reached. The curves show that at from 10 to 15 days after beginning to feed their young the body weight, in all groups, has returned to or below (3 groups) normal.

Food consumption at certain stages of the cycle. Current studies of this laboratory on the basal metabolism of these species at the various stages of reproduction give importance to information concerning possible variations in food consumption at various stages of the cycle. Food intake during the period of incubation (a period of increasing body weight) especially deserves attention, since it has been shown (1) that, when comparison is made with the "resting" stage metabolism, the basal metabolism of male doves is then decreased (that of females unchanged). We here record data on food intake at these two periods. Additional data, sufficient perhaps to differentiate the food intake of several races with differing metabolic rates, are being obtained with the aid of Guinevere C. Smith, and publication of details of nutritional data is reserved for that study.

For 4 pairs of pigeons and 1 pair of doves the "resting" stage was too short (following the feeding of young) to permit a satisfactory measure of the food intake at this stage. Among the remaining 24 pairs we find that 10 pairs (8 pigeons; 12 doves) consumed *more* food per day during the "incubation" (and "growth") stage than during the "resting" stage. Here the 4 pairs of pigeons showed an average daily food intake of 22.2 grams per bird during incubation and 19.2 grams during the "resting" stage. On the other hand, 14 of the 24 pairs (12 pigeons; 16 doves) consumed *less* food per day during the "incubation" (and "growth") stage than during the "resting" stage. These 6 pairs of pigeons had an average daily food intake of 20.3 grams per bird during incubation and 23.4 grams at the "resting" stage; for the 8 pairs of doves these values were 12.3 and 13.8 grams, respectively. These are the really significant values obtained from this series of measurements since they demonstrate that the increase in body size which occurs during incubation is very frequently accompanied by less food consumption than in the (non-growing) "resting" or copulatory stage. In other words, more often than not the current "total" metabolism (also the basal metabolism in males) is greater at the latter stage than during incubation. And if birds with stationary weight and higher food intake later accomplish an increase of weight on a diminished ration they probably do so by wasting less energy—by becoming relatively inactive—as the male pigeon very plainly does during incubation.

While parent pigeons are feeding young it is impossible to know how much grain is really assimilated by them and how much is regurgitated

into the mouths of their young. We nevertheless record the remarkable results of our measure of the average joint daily food consumption of one parent and of one young on the tenth and eleventh days after hatching. At this time the rapidly growing young is slightly less than half its adult size (2), and its parent is daily *losing* weight (see fig. 1); but together a parent and 1 young (pigeon) now consume 52.7 grams of food daily; the parent alone—while gaining weight during the last 6 days of "incubation"—consumed only 22.3 grams daily. A ring dove parent and 1 young ingest 22.9 grams daily, although the parent alone—in a period of size increase—ingested only 12.4 grams. It should be noted that even at this stage (10-day) in the "feeding of young" the parent birds are still losing weight although they spend much time brooding the young and are still relatively inactive. In other words, relative *inactivity* is a factor common to the period of size increase and to that of size reduction. The data last given show either that the parent birds are utilizing abnormally large amounts of food while losing weight and while relatively inactive, or that the young of 10 to 11 days are consuming amazingly large quantities—approximately one-fifth of their body weight—of solid relatively dry food. Other studies (3), (4) have shown that the highest point in the respiratory metabolism of the pigeon is attained at just this (10-day) period of life.

Comment. The slight increase in the body weight of females at the "ovulation" period is definitely explained by the great temporary hypertrophy (1000 per cent) of the oviduct under the influence of the female sex hormone, estrin. The much greater increase in body size here found to occur during "incubation" will also probably prove to be controlled by one or more incretions. This extra weight does not consist of fat; it is usually attained under normal or sub-normal volume of food intake, and under less than normal amount of exercise or activity. Nearly one-third of the increased weight at the very end of incubation is accounted for by the great temporary enlargement of the crop-glands which occurs at that time under the specific stimulus of prolactin (5). Though a measurable thickening of the crop-wall occurs only during the last half of incubation the increase of body size is apparent from the very beginning of incubation.

Concerning an incretory basis for the much larger fraction of the observed increase in body weight it can be stated that in doves and pigeons prolactin is released by the anterior pituitary (in detectable quantity) during all recurrent "incubation" stages and during that stage only in the whole life cycle of these birds; also that there is some evidence (Riddle, Bates and Lahr, unpublished) that this release of prolactin begins—as does the beginning of the increase in body size—at the beginning of incubation. Whether this cyclical release of prolactin is or is not accompanied by a simultaneous release of the growth hormone of the anterior pituitary, whether prolactin itself has growth-promoting power, or whether it is adjuvant to the thymocresin of Asher, or to still other growth-promoting factors,

are now matters of conjecture. Since this cyclic increase in body weight is restricted to reproducing adults the bones can have little share in it, but we see no real basis on which to differentiate the main fraction of this size increase from true growth.

The cyclic loss of weight by birds during the time they feed their young seems comprehensible in the following terms: The release of prolactin from the pituitary ceases probably soon after the feeding of the young begins; and growth influences—direct or indirect—which increased with the presence of prolactin in the blood would subside coincident with its absence. The crop-milk with which the young are fed during the first several days is formed at the expense of the parent bird's own tissue or body fluids, and the drain or strain on the parent body may be considerable—particularly since vitamines A and B are already known (6) to be lost from the parent in this crop-milk. Finally, it is conceivable that the temporary persistence of the bulky crop-glands during this period offers one or another impediment to an optimum intake or utilization of food.

SUMMARY AND CONCLUSIONS

The body weight of adult doves and pigeons undergoes a cyclic increase of about 8 per cent in weight during the 15 or 18 days spent in the incubation of their eggs. Maximum weight is attained at the end of incubation. Nearly one-third of this increase occurs in the crop-glands under the specific stimulus of prolactin. The remainder of the increased weight seems to occur in the body in general except in the skeleton, it is now indistinguishable from true body growth, and is believed to have a hormonal basis which in time relations at least is associated with the release of prolactin.

A cyclic decrease in the body weight begins as soon as parent birds begin to feed their young and continues for approximately 15 days. Probable causes of this loss of weight are considered.

The amount of food consumed by doves and by various races of pigeons during the several stages of the reproductive cycle was measured. For more than half of these birds it is found that the cyclic period of growth is a period of subnormal consumption of food—23 grams daily for a pigeon in the "resting" stage and 20 grams while incubating eggs.

At 10 to 12 days after hatching the young pigeon daily consumes a quantity of relatively dry grain approximately equai to one-fifth of its own body weight.

REFERENCES

(1) Riddle, O., G. C. Christman and F. G. Benedict. This Journal, 1933, **105**, 428.
(2) Riddle, O., D. R. Charles and G. E. Cauthen. Proc. Soc. Exper. Biol. and Med., 1932, **29**, 1216.
(3) Ginglinger, A. and C. Kayser. C. R. Soc. Biol., 1929, **101**, 711.
(4) Riddle, O., T. C. Nussmann and F. G. Benedict. This Journal, 1932, **101**, 251.
(5) Riddle, O., R. W. Bates and S. W. Dykshorn. This Journal, 1933, **105**, 191.
(6) Reed, L. L., L. B. Mendel and H. B. Vickery. This Journal, 1932, **102**, 285.

STUDIES IN THE INFLUENCE OF EXERCISE ON THE DIGESTIVE WORK OF THE STOMACH

I. Its Effect on the Secretory Cycle

FRANCES A. HELLEBRANDT and SARA L. HOOPES

From the Department of Physiology, University of Wisconsin, Madison, Wisconsin

Received for publication September 23, 1933

Work done in this laboratory (Hellebrandt and Miles, 1932) showed that exercise modifies the acidity of the gastric juice aspirated one hour after an Ewald meal. A better picture of the changes taking place in the stomach may be obtained by fractional analysis. No such reports have been found in the literature. The purpose of this experiment was, therefore, to extend the study already made on single samples to intermittent analysis during the whole of the digestive cycle.

Procedure. Repeated observations were made on one subject, a normal healthy young woman of twenty-one, a professional student in physical education accustomed to vigorous activity and giving no history of gastro-intestinal disease. After the usual morning program of classroom work the subject came to the laboratory having had no food or drink for about eighteen hours. A Rehfuss tube was swallowed and the fasting contents of the stomach aspirated. A Boas meal of 500 cc. of oatmeal gruel was ingested with the tube in situ. Subsequently the gruel was introduced through the tube to avoid all contaminations with saliva, being quickly admitted under gentle pressure from a flask connected to a source of compressed air. The speed of administration was regulated by varying the pressure with which the thumb was applied to one arm of the Y-tube through which the gruel passed from the flask to the stomach. Its automatic admission by this method was accompanied by no sensation. Saliva was continuously removed by suction. At 15 minute intervals 10 cc. samples of the gastric contents were removed until a negative starch test was obtained when iodine was added. This was considered the final emptying time of the stomach. Rehfuss (1927) believes that if the tube is properly placed, the disappearance of food from the aspirated contents may be taken as the end of digestion. The samples were withdrawn and forcibly re-introduced two or three times at each aspiration to secure a more representative specimen. After each withdrawal a syringeful of air was injected to empty the contents of the tube into the stomach. Since the acidity of the various parts of the gastric cavity varies, the same tube

was always swallowed to a measured point and there remained until adequate samples could no longer be obtained. This occurred toward the end of the digestive cycle. The tube was then raised or lowered as necessary, to insure complete emptying of the gastric contents. Note was made of the presence of bile in the samples withdrawn. These were titrated with N/10 NaOH using dimethylaminoazobenzene and phenolphthalein as indicators. Total chlorides were determined by the method of Volhard.

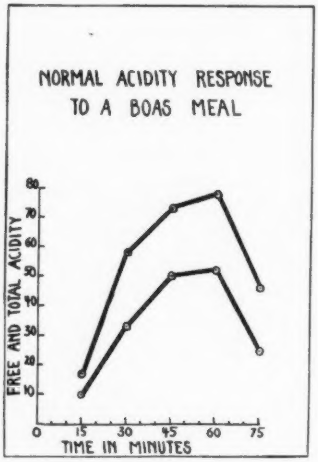
Studies were made on the influence of three types of exercise, short violent activity, protracted and exhaustive muscular work, and mild exertion. Their effects were observed both when they preceded and followed the test meal. The first type of exercise lasted only three minutes and was exceedingly rigorous, being performed at as high speed as the subject could maintain when putting forth her maximum physical effort. It was divided between running in place, turning a hand ergometer, deep knee bending alternating with a jump, and pedalling a stationary bicycle. Four successive periods, working 15 minutes on the rowing machine, 20 on the bicycle ergometer, 10 on the hand ergometer and 15 on the treadmill, constituted the protracted exercise. The activities were administered in batteries because the variation had a good psychic effect. It prevented stereotyping. The subject redoubled her efforts without being driven as her interest was aroused by the introduction of a new exercise. During both of these batteries the subject was flushed, sweating and dyspneic, being near the point of exhaustion at their completion. The mild exercise consisted of walking out-of-doors for one hour at a leisurely pace.

Three series of experiments extending over a period of six months were performed on the same subject. Confirmatory observations were made upon another young adult woman, a very active participant in sports. She was nervous and high strung and less robust than the first subject, although apparently in good health.

Results and their interpretation. Studies based on observations made by means of fractional analysis after a test meal have been criticized as being variable and in consequence unreliable. Kopeloff (1922) found as great variance in the same individuals as among different subjects. However, Bell and MacAdam (1924) observed one man for twenty consecutive days and report that the emptying time was remarkably constant and that the type of curve remained the same, even though the variation in individual acidities was great. In interpreting the data obtained in our experiments, a general constancy in the response of a single person to identical stimuli is being assumed, and emphasis is placed on the contour of the secretory curve as a whole rather than upon comparative acidities at specified times in the cycle.

The accompanying graphs reveal certain interesting trends. The normal curve obtained at rest and used as a standard for comparison (graph 1)

tends to be symmetrical with a slightly more deliberate rise in acidity than descent. The peak acidity is higher and the emptying time is shorter than the average normal as determined on young men by Bennett and Ryle (1921) and by Baird, Campbell and Hern (1924). Violent exercise (graph 2) retarded and prolonged the cycle. Protracted exercise (graph 3) induced an initial total suppression of free HCl followed by an abrupt rise, the peak occuring later than normal and the emptying time being markedly delayed. The effects of both types of severe exercise decreased as the subject became accustomed to the activity. Mild exercise exhibited no inhibiting influence on the secretory response (graph 4). It prolonged the emptying time somewhat and, as illustrated, occasionally augmented secretion slightly.

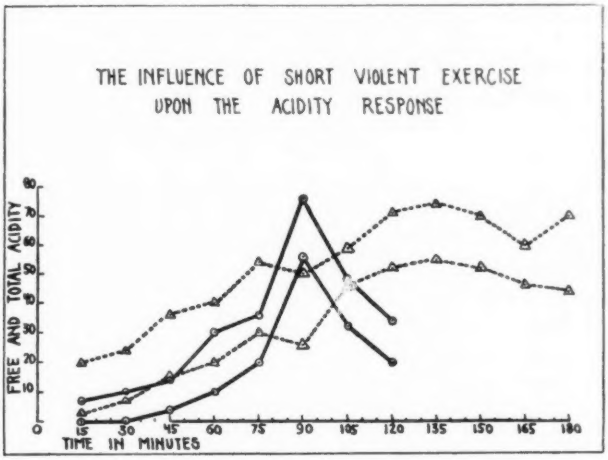


Graph 1. The secretory response obtained with the subject at rest. The upper curve represents total acidity and the lower, free HCl.

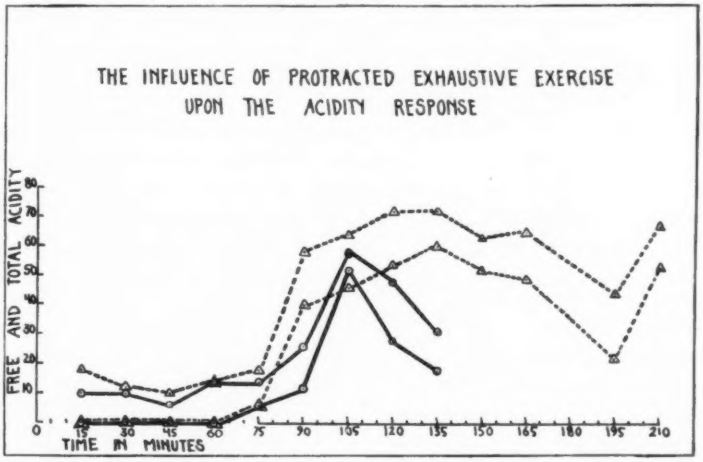
All types of exercise indulged in subsequent to the ingestion of the test meal prolonged final emptying time more than the same activity preceding the meal, and the more stringent the exertion, the greater the delay. It was frequently observed that the early anacidity or hypoacidity might eventually be supplanted by a period of secretory augmentation with an acme significantly higher than normal (graph 5).

The observations on the second subject were similar except that exercise occasionally hastened the final emptying time. During the course of the corroborative experiments, this subject gradually developed an anacidity unassociated with disease and could no longer be considered as satisfactory for experimental purposes. This interesting phenomenon is being further studied and will be reported subsequently.

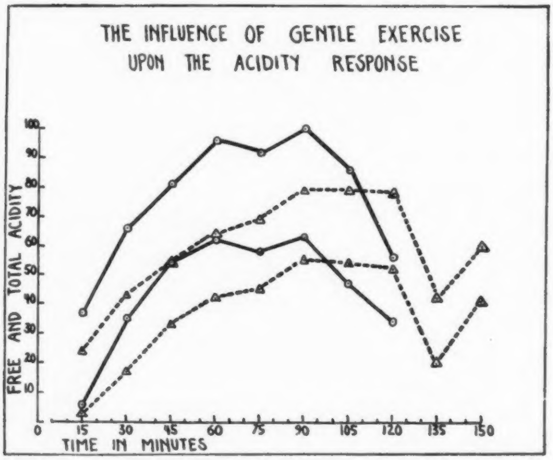
Discussion. The findings do not necessarily imply that the effect of severe exercise is to inhibit the secretory work of the gastric glands. Certain mechanical and chemical factors may modify the acidity of the gastric content independent of any alteration in the rate of secretion, or in the concentration of the constituents from which it is composed. Babkin (1927) presents three mechanisms capable of reducing gastric acidity. Dilution and neutralization secondary to contamination with saliva is not ordinarily, but may be, a factor of importance. Continuous intubation is sometimes associated with excessive salivation, a liter of saliva being col-



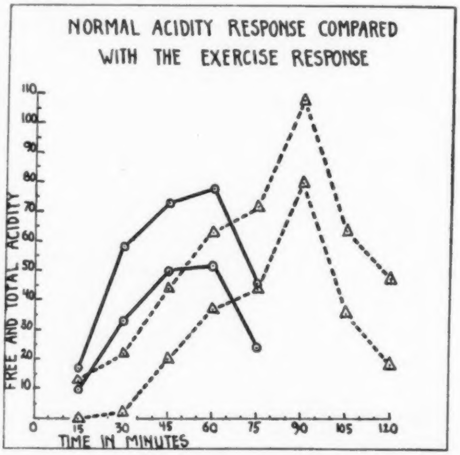
Graph 2. The violent exercise lasted for three minutes and was divided between running in place, turning a hand ergometer, deep knee bending alternating with a jump, and pedalling a stationary bicycle. The solid lines represent the acidity response when the exercise preceded the meal, the broken lines, when it followed the meal. The upper curves represent total acidity, the lower, free HCl.



Graph 3. The protracted exhaustive exercise lasted for one hour and was divided between work on the rowing machine, bicycle ergometer, hand ergometer and treadmill. The solid lines represent the acidity response when the exercise preceded the meal, the broken lines, when it followed. The upper curves represent total acidity, the lower, free HCl.



Graph 4. The mild exercise consisted of walking out of doors at a leisurely pace for one hour. The solid lines represent the acidity response when the exercise preceded the meal, the broken lines, when it followed. The upper curves represent total acidity, the lower, free HCl.



Graph 5. This shows the tendency for exercise to induce an initial hypoacidity followed by a delayed augmentation of the secretory response. The solid lines represent the normal, resting response, the broken lines, the influence of protracted exercise upon it. The upper curves represent total acidity, the lower, free HCl.

lected during a two and an half hour digestive cycle. Salivary secretion was effectively removed by suction and distortion of the acidity curve from this source was negligible. The gastric mucus is alkaline. The HCl may be neutralized by it during gastric digestion but Babkin considers the part it plays of no great consequence. Regurgitation of duodenal juices is of greater importance. Reflux from the intestine as grossly detected by the presence of bile never occurred early. An anacidity or low HCl do not imply that the gastric secretion is correspondingly reduced. The determination of total chlorides must be resorted to for the differentiation of true achylia from a low acid due to excessive neutralization. The total chlorides closely followed the acidity curve except late in the cycle when they diverged, remaining high while free and total acidity fell. These findings indicate that neutralization by regurgitated duodenal contents played no part in the early malconformation seen in the acidity curves when distressing exercise was associated with the digestion of the test meal.

The great delay in the final emptying of the meal as measured by the disappearance of starch shows that the motor power of the stomach was strikingly affected by exercise. In general, the more severe the muscular exertion the longer the emptying time. This is important. If the speed with which chyme is ejected into the duodenum is inhibited, then the retained gruel will dilute the acid juice being secreted into it and thus lower the acidity curve. The secretion of the same juice into a steadily diminishing gastric volume will be associated with a steady rise in acidity. Only if the secretion is totally inhibited will free HCl be absent from the aspirated samples. An hypoacidity may therefore mean either secretory inhibition or gastric atony. The mechanism of the modification of the secretory curve cannot be determined without further information concerning the influence of exercise upon the motor behavior of the stomach. Its estimation by the disappearance of starch is inadequate because it pictures only final emptying, and throwing no light upon the time of occurrence and the duration of the inhibition, its distorting effect upon the curve of secretion cannot be determined.

SUMMARY AND CONCLUSIONS

The effects of three different types of exercise on the gastric secretory cycle were studied by continuous intubation and fractional analysis. It was found that:

1. Severe and exhausting exercise inhibited the initial secretory response, being associated with an hypoacidity or anacidity lasting as long as one hour.

2. The initial inhibition was at times superseded by transitory hyperacidity.

3. Mild activity increased or left the peak acidity unchanged.

4. All types of exercise prolonged the digestive cycle as measured by the disappearance of starch, the delay in final emptying being more marked when the exercise followed the meal, and greater in proportion to the severity of the exercise.

5. In general the effects of exercise on the gastric secretory cycle decreased as the activities were repeated.

6. A dependence of low acidity upon decreased motility is suggested.

REFERENCES

Baird, M. M., J. M. Campbell and J. R. B. Hern. 1924. Guy's Hosp. Rept., **74,** 339.

Bell, J. R. and W. MacAdam. 1924. Amer. Journ. Med. Sci., **168,** 520.

Bennett, T. I. and J. A. Ryle. 1924. Guy's Hosp. Rept., **71,** 286.

Hellebrandt, F. A. and M. M. Miles. 1932. This Journal, **101,** 258.

Kopeloff, N. 1922. Journ. Amer. Med. Assn., **78,** 404.

Rehfuss, M. E. 1927. Diagnosis and treatment of disease of the stomach with an introduction to practical gastro-enterology. Philadelphia.

STUDIES ON THE INFLUENCE OF EXERCISE ON THE DIGESTIVE WORK OF THE STOMACH

II. Its Effect on Emptying Time

FRANCES A. HELLEBRANDT and RUBYE H. TEPPER

From the Department of Physiology, University of Wisconsin, Madison, Wisconsin

Received for publication September 23, 1933

The influence of exercise upon gastric motility has been fluoroscopically studied with conflicting results by Nielsen (1922), Dickson and Wilson (1924) and by Kasabach (1931). Their findings indicate that mild activity either hastens emptying time (Nielsen) or has no appreciable effect upon the motor power of the stomach (Dickson and Wilson). Relatively strenuous exercise, long time jogging, was also observed to shorten emptying time (Kasabach), but violent running invariably inhibited gastric peristalsis (Dickson and Wilson). We have reason to believe that the influence of exercise upon the gastric secretory response may be incumbent upon concomitant motility changes (Hellebrandt and Hoopes, 1934). Our purpose was, therefore, to measure quantitatively the influence of exercise upon the motor power of the stomach during the whole of the digestive cycle and thus elucidate this point.

Procedure. A modification of the method of Kaufman and Lipkin (1932) was used for the study of gastric emptying time. Farina was selected as the vehicle for the barium sulphate. The major observations were made after the ingestion of 250 cc. of thick porridge composed of 30 grams of farina cooked down in 400 cc. of water, after which 150 grams of barium sulphate were added. The meal was salted to taste and thoroughly mixed with an electric beater. The subject stood in a comfortable position in front of a vertical fluoroscope with the right arm and shoulder against a perpendicular attached to the apparatus for the purpose of aiding in the maintenance of a constant posture. The position of the feet was outlined on the floor. The meal was administered warm and eaten with a spoon as rapidly as possible. The interval between the deglutition of the first mouthful and the opening of the pylorus was measured with a stopwatch and called the *initial emptying time.* Immediately after the first ejection of barium and farina into the duodenum and the completion of the ingestion of the meal, an outline of the stomach shadow was drawn upon the screen. So that the position of the latter could be replaced at subsequent observations, it was measured by means of pointers moving with the

screen against meter rules attached vertically and horizontally to the supporting framework. For permanent record the outline of the shadow was transferred to paper. All except the greater curvature was then erased from the screen, this serving as a landmark. Observations were made sixty minutes after the beginning of the meal, thirty minutes later, and henceforth at quarter hour intervals until the gastric shadow was no longer visible. The interval between the beginning of the meal and the last visible shadow was called the *final emptying time.* The shrinkage of the shadow visualized fluoroscopically was measured with a planimeter, the resulting data, calculated in terms of percentage of the initial size, being the basis for the construction of a curve of gastric emptying.

The subjects were young women ranging in age from nineteen to twenty-three, free from gastro-intestinal symptoms or disease, professional students in physical education who had much and regular exercise. The activities studied were classified as violent, exhaustive and mild and were administered as described in the first paper (Hellebrandt and Hoopes, 1934). Observations were made twelve to eighteen hours after the last meal and once the porridge had been given, no eating or drinking was permitted until final emptying occurred. Between examinations the subjects sat quietly in the fluoroscopic room.

To be adequately accommodated the operator remained in the dark room for at least 20 minutes before the first scrutiny. When normal observations were made or exercise followed the meal, the subject rested in the fluoroscopic room during this period of accommodation so that basal conditions might be attained. The subjects were examined only in the antero-posterior position using discontinuous short periods of observation and minimal exposure. For their protection, examinations were made no more frequently than once in twenty-one days. Each subject was observed at least five different times between the months November and April. We were able to find no data on the constancy of the form, motility, position and contour of the stomach upon repeated examination under identical conditions. Because we did not know whether a standard for comparison obtained six months prior could still be accepted, or whether the response of the stomach is too changeable to justify such a procedure, control observations were made upon two subjects.

Results and their interpretation. Thirty-six fluoroscopic examinations were made on seven different subjects. Figure 1 shows the outline of the first gastric shadow made on one of them at five monthly intervals. The first three were drawn after the ingestion of the standard meal; the last two, after the meal was reduced to one-half and three-quarters of this amount respectively. Because of nausea, subjects were sometimes unable to eat the entire meal. This procedure was followed to discover how a reduction in meal size modified the behavior of the stomach. The shad-

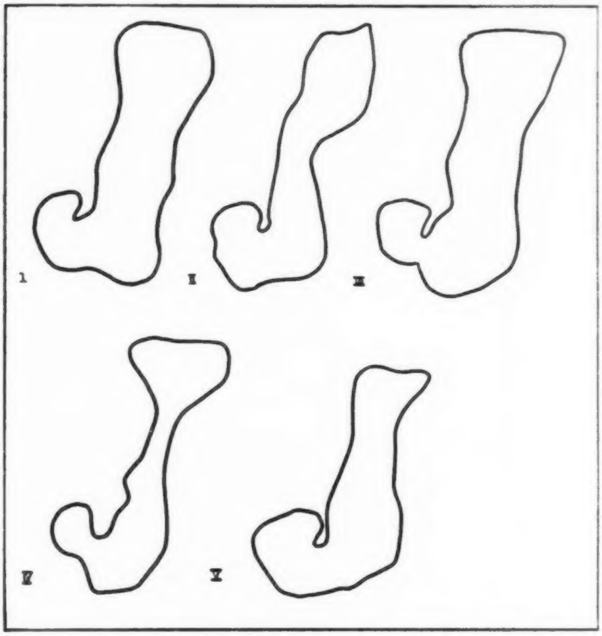
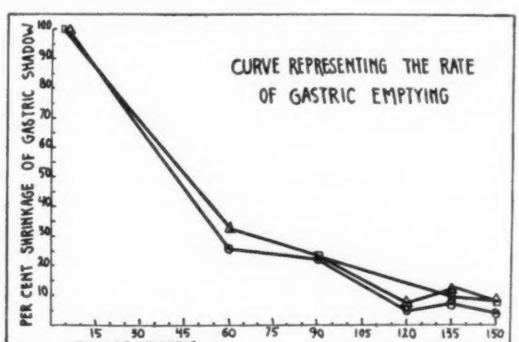
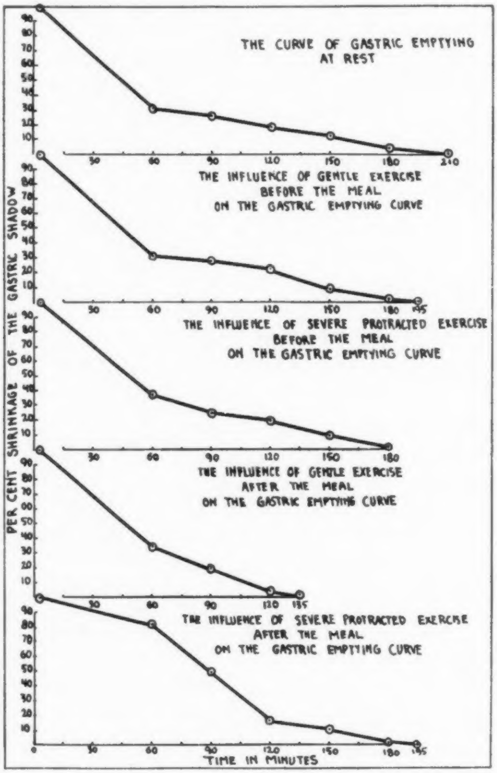


Fig. 1. The gastric shadow of one subject drawn at monthly intervals immediately after the ingestion of the test meal and initial emptying time. The meal was reduced by ½ at IV and ¾ at V.



Graph 1. The three curves were obtained on the same subject under conditions of rest at monthly intervals. They commence at the initial and end at the final emptying time.

ows show a striking resemblance to each other; the type of stomach, the shape and the contour of the outlines are all similar. Graph 1 shows as remarkable a similarity in the rate of emptying of the same stomach with the subject at rest after the ingestion of the standard meal at three monthly intervals. Final emptying time was identical. Similar observations were

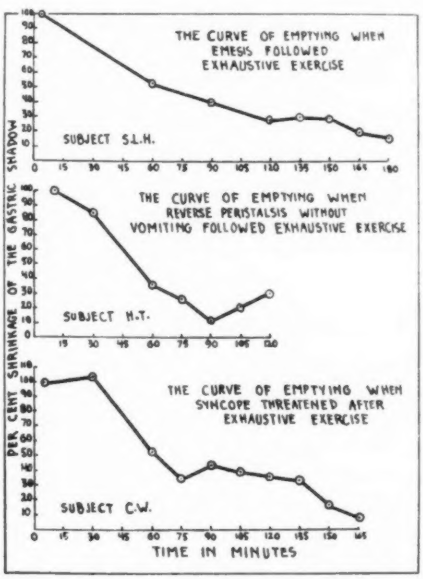


Graph 2. Curves of gastric emptying time at rest and following exercise. The composite response of five subjects. The exercise lasted for one hour.

made on the other control. There seems to be a characteristic gastric response to this meal and under the same conditions the motor reaction of the stomach to it remains constant.

Graph 2 shows the average resting curve of gastric emptying based on the size of the stomach shadow of five subjects, and the influence of severe and mild exercise upon it. At rest, evacuation occurs most rapidly during the first hour immediately following the ingestion of the meal. Occasion-

ally the meal was observed to collect high in the fundus and only gradually trickle in a narrow stream to the lower pole of the stomach. Peristalsis normally came on quickly. Not infrequently the first portion of the meal passed into the duodenum before the full quota had been eaten. The average initial emptying time at rest was four minutes and ten seconds. The opaque meal was sometimes seen to enter the duodenum in less than two minutes after the first spoonful had been swallowed. At the sixty minute observation the peristaltic waves were usually deep and powerful. The emptying was more deliberate during the second hour. The rate remained slow until final emptying occurred, on an average in $3\frac{1}{2}$ hours.



Graph 3. Curves showing individual reactions of the motor power of the stomach to exhaustive exercise.

Gentle exercise tended to hasten gastric emptying, particularly if indulged in after the meal. Severe exercise likewise slightly hurried the final emptying of the contents of the stomach into the duodenum. When it preceded the meal it had no great effect upon the contour of the curve of emptying, but following immediately upon its ingestion was markedly inhibiting. During the period of exercise little emptying occurred. When examined immediately after the cessation of muscular activity, the stomach either appeared totally inactive or peristalsis was feeble and shallow. Recovery was usually prompt and emptying was greatly accelerated during the second post-exercise hour.

Protracted exercise either hastened or delayed emptying time depending upon the reaction of the subject to the muscular exertion, interesting variations being obscured in the average curve presented. Three of these are recorded in graph 3. Subject S. L. H. vomited when the meal was eaten immediately after the severe and protracted bout of exercise. Roughly one-half of it was lost by emesis before the first shadow could be drawn. In spite of this three hours elapsed before final emptying occurred, and as may be seen from an examination of the curve, the delay was not confined as normally to the first post-exercise hour. Subject H. T. found the meal especially disagreeable. When ingested after exhausting exercise she was unable to complete the standard portion. The stomach filled like a balloon. It was smooth and inactive. Vigorous reverse peristalsis set in after the first few mouthfuls and continued until eating was stopped. The shadow in the region of the antrum was large and smooth. The subject made no complaint and upon questioning reported no nausea. She was extremely fatigued, leaning heavily against the apparatus, so relaxed that it was difficult to maintain the upright posture. Ten minutes and fifty seconds elapsed before initial emptying occurred. Because of the peculiar behaviour of the stomach it was reëxamined in 30 instead of 60 minutes. Slow deep peristaltic waves were sweeping over the stomach from fundus to antrum, but as the curve shows, little emptying had occurred. From henceforth the behavior was not much different than normal. Subject C. W. was also very fatigued after a similar battery of exercises. She reported nausea with the meal but ate the full portion. The stomach was round, smooth and passive, sinking as each bolus dropped into the viscus. It swelled uniformly under the influence of gravity and its walls appeared to offer no resistance to the meal. Four and three-quarter minutes after the beginning of eating the subject complained of vertigo. The pulse was small, thin and hurried. As it grew feebler the subject was placed in the recumbent position to avert syncope. Loss of vasomotor tone in the splanchnics with resulting cerebral anemia was the cause of this phenomenon. Recovery was uneventful and the experiment was completed. As may be seen from the curve of emptying, gastric paralysis occurred during the first half hour. At the end of this time the gastric shadow was larger than it had been immediately after the ingestion of the meal. This may have been due to excessive salivation or the secretion of mucus, these being added to the contents already trapped within an atonic stomach, or to reflux from the duodenum.

A meal of 150 grams of barium sulphate added to 50 grams of farina cooked in 500 cc. of water was given six times to one subject. Final emptying for this meal averaged five hours, during the whole of which time intermittent observations were made in the usual way. Figure 2 is a reproduction of the size of the gastric shadow immediately after the inges-

tion of this meal and its initial emptying, one hour, and finally two hours later. The first series of three was taken with the subject at rest, the second when three minutes of violent exercise followed the meal, and the last when the protracted battery of exercises were performed after the meal.

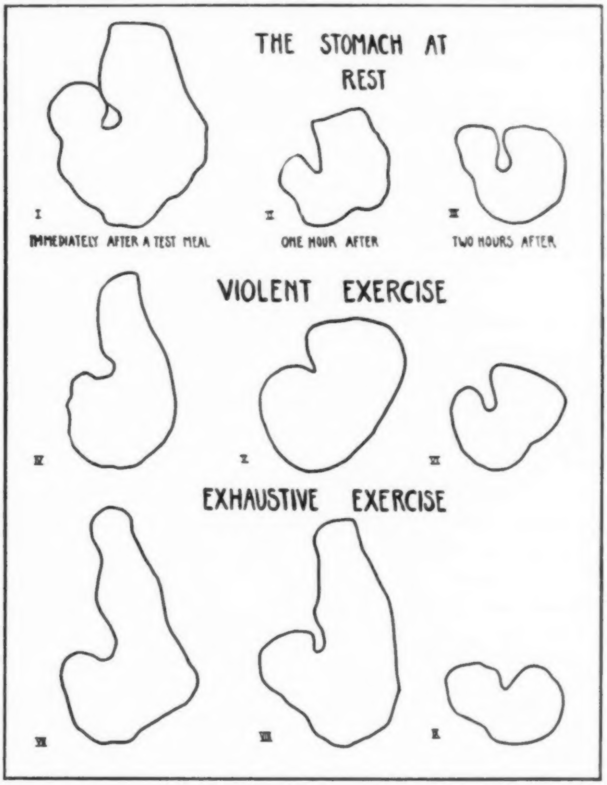


Fig. 2. Sketches showing the influence of violent and exhaustive exercise upon the size of the gastric shadow one and two hours after the ingestion of a test meal and initial emptying time.

Neither of the severe exercises could be comfortably executed because of the size of the meal and the mechanical obstruction to the free descent of the diaphragm which it afforded. Both were associated with gastric paresis. The area of the stomach shadow one hour after the meal was larger than it had been immediately following its ingestion. Emptying occurred at an accelerated rate during the second post-exercise hour, so that

120 minutes after the beginning of the observations the relative size of the shadows was not much different than normal.

The individual curves of gastric emptying not infrequently reveal a retrograde phenomenon late in the digestive cycle. This is probably caused by duodenal regurgitation. Its appearance corresponds in time to the divergence of the chloride and acidity curves in the study of gastric secretion. When observing the secretory response to a gruel meal, all types of exercise tended to delay the completion of the digestive cycle and prolong the disappearance of starch from the aspirated samples. No such inhibition in final emptying was demonstrated by fluoroscopic examination. Curiously enough, the subject upon whom the major secretory studies were performed responded in this ambiguous way when observed by the two methods. Obviously the determination of motility by a method permitting simultaneous acidity studies would be of value.

SUMMARY AND CONCLUSIONS

The most interesting points demonstrated from a fluoroscopic study of the influence of exercise on gastric motility were first, that the most profound motor changes occur, like the secretory ones, during the first third of the digestive cycle; and second, that like the secretory response the phase of inhibition may be followed by a period of augmented activity. The ultimate task to be accomplished by the stomach is completed in much the usual way. It seems merely to stand still during the muscular activity or the period of immediate post-exercise recovery. Once readjustments occur, it picks up the work it must perform and proceeds as though nothing had happened, perhaps even more briskly than if exercise had not stimulated the general metabolism. In the end, exercise closely associated with meal-time does not seem detrimental as far as digestion is concerned. It delays it, but the transitory inhibition appears to do no harm. This may be an illustration of one of the beautiful adaptive mechanisms which play so important a part in the coöperative bodily response to exercise. It remains to be said that if exercise follows immediately upon the ingestion of a full meal, the gastric atony may not have so inconsequential an effect upon the ease and skill of the performance of that exercise. Its modifying effects are possibly of greater importance to athletic performance than to the general body economy. Suffice it to say the following:

1. The form, shape and contour of the stomach and its motor response to an opaque meal are remarkably constant.

2. Mild exercise tends to hasten final emptying time, especially if it follows immediately upon the ingestion of the meal.

3. Violent and exhaustive exercise inhibits gastric peristalsis, but this may be followed by a period of augmented activity so that final emptying is not much altered.

4. Retrograde phenomena frequently appear late in the digestive cycle.

5. The time of occurrence of motility changes corresponds to the time of occurrence of the secretory variations already reported.

REFERENCES

Dickson, W. H. and M. J. Wilson. 1924. Journ. Pharm. Exp. Therap., **24**, 33.

Hellebrandt, F. A. and S. L. Hoopes. 1934. This Journal, in press.

Kasabach, H. H. 1931. Arch. Int. Med., **48**, 1237.

Kaufman, L. W. and S. S. Lipkin. 1932. Unpublished thesis, University of Wisconsin.

Neilsen, A. A. 1921. Acta Radiol., **1**, 379.

STUDIES IN THE INFLUENCE OF EXERCISE ON THE DIGESTIVE WORK OF THE STOMACH

III. Its Effect on the Relation between Secretory and Motor Function

FRANCES A. HELLEBRANDT and LYNDALL L. DIMMITT

From the Department of Physiology, University of Wisconsin, Madison, Wisconsin

Received for publication September 23, 1933

Few simultaneous acidity and motility studies have been made to determine if a relation exists between the secretory and motor functions of the stomach. Carlson (1915a, b) had observed that in the empty stomach of his subject with gastric fistula the secretion of gastric juice is continuous, has a practically constant total acidity, varies in rate, and thus has an acidity proportional to its rate. At this time Carlson suggested that the most important factor in the variability of the volume of the contents of the fasting stomach was probably the tonicity of the viscus and its consequent rate of emptying. Later (1916) he noted that the mucin content of the juice increased whereas the free HCl decreased during strong hunger contractions. In 1925 Hoelzel re-studied the problem in a normal man and came to the conclusion that on the whole acidity is higher during activity than quiescence. We have presented evidence suggesting that exercise variations in gastric acidity may be related in large part to changes in the motor power and the rate of emptying of the stomach (Hellebrandt and Hoopes, 1934; Hellebrandt and Tepper, 1934). Further, we have reason to believe that the initial inhibition and secondary augmentation in functional capacity affects both forces simultaneously. Objective proof of these assumptions would throw considerable light upon the mechanism of the effects of exercise on the digestive work of the stomach. The purpose of this experiment was therefore, to make concurrent studies of gastric acidity and motility under conditions of rest and muscular activity.

Methods. The acidity and motility observations of Hoelzel on the fasting stomach were not strictly simultaneous. He alternated the introduction of the stomach tube and the balloon, removing the tube to minimize mechanical stimulation and withdrawing the balloon to facilitate complete aspiration. As a result of extensive experience with continuous intubation, Rehfuss (1927) believes that the presence of a small tube in the stomach has little distorting effect upon the secretory response of that organ. Two standard Rehfuss tubes were therefore cemented together, with

the tip of one falling about 10 cm. below the other. The metal olive was removed from the shorter tube and replaced by a delicate thin walled rubber condom. The balloon was connected with a Marey tambour through a water manometer.

All of the observations were made upon one subject and extended over a period of six months. The subject was a normal healthy young adult woman accustomed to strenuous physical activity and to gastric intubation. She swallowed the double tube without difficulty and maintained it for many hours without subjective sensation or discomfort. Observations were commenced 14 to 20 hours after the last meal. They were preceded by a half-hour period of rest in the semi-recumbent position in a steamer chair, so that basal conditions might first be attained. The double tube was always swallowed to a given mark at the beginning of the rest period and remained undisturbed throughout the entire procedure. Continuous pneumographic and intragastric pressure records were made upon a slowly moving kymograph. The subject remained quietly in the experimentation room until hunger contractions appeared. A small Boas meal of 100 cc. of strained oatmeal gruel was then introduced through the tube to avoid contamination with saliva. Salivary secretion was removed by suction. At 5 minute intervals 2 cc. samples of the gastric contents were withdrawn, first mixing thoroughly by aspirating and re-introducing a full syringe of the fluid two or three times. After the removal of each sample the stomach tube was emptied by the introduction of air and the syringe was rinsed in distilled water. Each sample was tested for starch with iodine, and free and total acidity were determined by titration with N/100 NaOH. Observations were continued until hunger contractions reappeared. For the study of the influence of exercise a variety of activities was used, including the violent, exhausting and mild bouts described in the first paper (Hellebrandt and Hoopes, 1934). The severe exercises were poorly tolerated when they followed the introduction of the meal and were attempted with the tubes in situ. The bodily movement so marred the records of intragastric pressure that they were impossible to interpret once they had been obtained. The most satisfactory results were gotten when the subject performed exercise limited to pedalling a stationary bicycle ergometer. She could move from her position of rest to the exercise machine and back again without being disconnected from the recording apparatus.

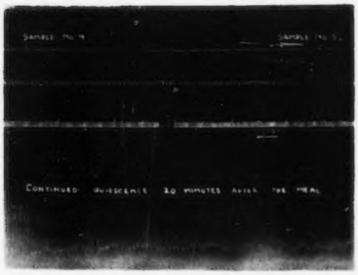
Results and their discussion. Powerful rhythmical contractions usually made their appearance within a half-hour after the tubes were swallowed. Occasionally a prolonged period of relative quiescence was met with, three or more hours elapsing before the onset of intragastric pressure changes indicating peristaltic activity. The so-called hunger contractions were normally very powerful. They usually terminated in vigorous incomplete tetanus. The period of activity lasted an average of 24

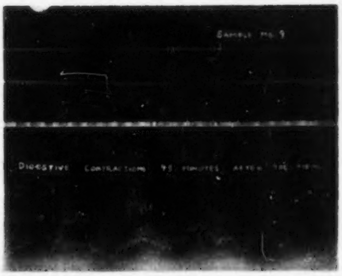
minutes, varying in duration from 14 to 49. During this time an inconstant number of contractions occurred, ranging from 9 to 31 and averaging 17. The culminating tetany usually lasted for about 3.5 minutes. The cessation of activity was always abrupt. These findings are in good agreement with those of Carlson (1916).

The meal was never introduced before hunger contractions were first well established. Its administration was invariably followed by a prompt cessation of activity. Whether the meal was introduced whilst a hunger contraction was in progress, in the interim between contractions or during the culminating tetanus, evidences of motility quickly subsided. Relative quiescence varying in duration from 13 to 30 minutes followed the introduction of the gruel. Its average duration in response to the small meal used was 21.5 minutes after which distinguishable tonus variations supervened. A tonus rhythm of great uniformity was established, lasting on an average slightly longer than the quiescence which immediately preceded it. These tonus changes subsequently increased in intensity until they merged into the strong contractions characteristic of the onset of a new hunger period. This occurred on an average 47 minutes after the reception of 100 cc. of gruel by the fasting stomach. The sequence of the intragastric pressure changes observed on this young woman are much like those described in 1915 by Rogers and Hardt.

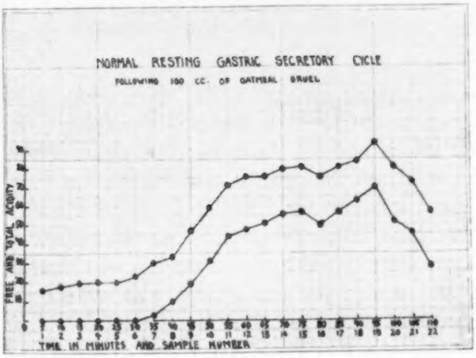
When the gastric acidity data are matched with the kymographion records simultaneously obtained, a striking unison of response is evident. The parallelism of the secretory and motor changes is beautifully seen in the illustration presented (fig. 1). During the period of motor quiescence following the introduction of the meal successive samples of the gastric contents contained no free HCl, and the value of the total acidity maintained a plateau. The onset of the tonus rhythm was associated with the initial appearance of free HCl and the rise of total acidity. Midway between the aspiration of the 15th and 16th samples the "digestive contractions" disappeared and a transitory phase of quiescence ensued. During this time specimens were aspirated with difficulty, after which the fluid again became more abundant. There was a slight and transitory fall in acidity. Strong contractions characteristic of the onset of a hunger period came on just prior to the acme of the secretory cycle. At this time the iodine test for starch was still positive. The period of strong rhythmic contractions lasted for 14′ 21″. There were 9 hunger contractions which culminated in a tetanus lasting for 8′5″. *It was during the period of gastric tetany that the first starch negative sample was withdrawn.* These phe-

Fig. 1. Reproduction of the motility and acidity response of the stomach to a small gruel meal, with the subject at rest. The records were simultaneously obtained by double intubation. Corresponding points are marked on the kymograms and the secretory curve.


SAMPLE NO. 4
SAMPLE NO. 5
CONTINUED QUIESCENCE 20 MINUTES AFTER THE MEAL


SAMPLE NO. 9


RETURN OF HUNGER CONTRACTIONS AND TETANUS


NORMAL RESTING GASTRIC SECRETORY CYCLE
FOLLOWING 100 CC. OF OATMEAL GRUEL
FREE AND TOTAL ACIDITY
TIME IN MINUTES AND SAMPLE NUMBER

nomena were repeatedly observed. We are convinced that they are not accidental. Starch negative samples were never obtained before the onset of unmistakable "hunger contractions." They appeared during the hunger contractions, the tetanus which marked the end of the period of strong contractile activity, or early in the quiescence into which it abruptly lapsed.

In the light of the above, the term "hunger contractions" is a misnomer. We observed them to occur while the stomach still contained the residues of the meal we had introduced. Rogers and Hardt (1915) made a similar observation and considered it significant that although it was not always the case, the first contractions felt by the subject might occur while the stomach still contained food. It is not intended to imply that the bodily state of hunger may not influence the mechanism concerned in the control of gastric tone and motility.

When exercise either preceded or followed the introduction of the test meal, no characteristic and uniform alterations could be detected in the form, amplitude or rate of the contractions of the stomach. Only one phenomenon repeatedly appeared, quiescence following the introduction of the meal was never noted after a bout of exercise. It is to be remembered that the muscular exertion was limited to relatively mild activity because of technical difficulties. Quite uniformly the tonus rhythm came on at once. The parallelism between the secretory and motility response noted in the resting state continued during exercise. The milder types of work on the stationary bicycle tended to heighten the acidity and shorten the cycle. More severe exercise on the ergometer, especially if it followed the meal, lowered the acidity, prolonged the appearance of hunger contractions and the disappearance of starch. These results are essentially in accord with those reported in the first two studies and substantiate the assumption that it is reasonable to explain the secretory curve inhibition in part at least, in terms of reduction in the rate of gastric evacuation. Because of the limitations imposed by the method we were unable to obtain direct evidence of unison in response during violent and exhaustive exercise, but there is no reason to suspect that this does not follow.

Comment. The findings give presumptive evidence that the mechanism of the change in gastric function induced by exercise must be the same for secretory and motor power. Carlson (1916) has shown that the fasting stomach isolated from the central nervous system behaves much like the one with its extrinsic nerves intact. It follows that the mechanism controlling the muscular activity of the stomach resides in the intrinsic innervation of its wall. The ultimate function of the stomach is to discharge into the duodenum the substances with which it is periodically loaded. Our results suggest that the "hunger contractions" may be the augmented efforts of an almost empty stomach to rid itself of the last resi-

dues of the foodstuffs which had been introduced. Under the fluoroscope one may visualize the same phenomenon. For some time before the disappearance of the gastric shadow cast by an opaque meal, the deep and deliberate peristalitic waves which characterize its motor conduct during most of the period of emptying, are replaced by a much augmented activity. Once the last remnants of a test meal disappear from aspirated gastric samples, the secretory and motor activity both subside. It is conceivable that accumulations of the gastric juice being continually secreted at low rate in the inter-digestive phase, or accumulations of fluid regurgitated from the duodenum into the relatively quiescent stomach may, through a local mechanism, bring on another series of "hunger contractions." They are known to occur at irregular intervals. They are always preceded by an augmented tonus rhythm. Large accumulations of fluid have been found at the end of quiescent periods (Hoelzel, 1925). The bulk of the fluid has been observed to leave quickly with the onset of tonus rhythm and before definite gastric contractions come on (Hoelzel, 1925). The "hunger contractions" may therefore represent the method the stomach takes of emptying itself of the last vestiges of an accumulation of fluid.

SUMMARY

Simultaneous gastric acidity and motility observations made under conditions of rest and muscular activity show a striking parallelism in response. Variations in the rate of evacuation must therefore be considered in any explanation of the changes in acidity observed. The findings suggest that the mechanisms controlling the secretory and motility change in response to a meal must be identical.

REFERENCES

Carlson, A. J. 1915a. This Journal, **37**, 50.
1915b. This Journal, **28**, 248.
1916. The control of hunger in health and disease. Chicago.
Hoelzel, F. 1925. This Journal, **73**, 463.
Rehfuss, M. E. 1927. Diagnosis and treatment of diseases of the stomach with an introduction to practical gastro-enterology. Philadelphia, 270.
Rogers, F. T. and L. L. J. Hardt. 1915. This Journal, **38**, 274.

STUDIES IN THE INFLUENCE OF EXERCISE ON THE DIGESTIVE WORK OF THE STOMACH

IV. Its Relation to the Physiochemical Changes in the Blood

FRANCES A. HELLEBRANDT, HARRY D. BAERNSTEIN and SARA L. HOOPES

From the Departments of Physiology and Physiological Chemistry, University of Wisconsin, Madison, Wisconsin

Received for publication September 23, 1933

We have already demonstrated that exercise, especially if it be violent or exhaustive and if it follow immediately upon the ingestion of a test meal, produces an hypoacidity or an anacidity which may last for more than one hour (Hellebrandt and Hoopes, 1934). We have also presented evidence for the belief that the concomitant gastric atonia is responsible, in part at least, for the abnormally low acidity of the stomach contents (Hellebrandt and Tepper, 1934). We have found that the acidity of the gastric juice is not much affected during the phase of abnormal reaction by neutralization and dilution secondary to duodenal regurgitation. This is the automatic mechanism which plays the most important part in the control of the acidity of the gastric contents under conditions of rest (Boldyreff, 1915; Stewart and Boldyreff, 1932).

In 1924 Dickson and Wilson suggested a relation between the peristaltic activity of the stomach and changes in the acid-base equilibrium of the blood. The effects of experimental acidosis and alkalosis led them to the conclusion that the activity of the gastric musculature is dependent upon the tension of carbon dioxide in the arterial blood. Studying the effects of exercise, they observed as we did, that if the activity be violent enough, peristaltic inhibition occurs. They reasoned that the chief factor in this delay was probably due to an increase in hydrogen-ion concentration secondary to the accumulation of lactic acid, but offered no experimental data on this point.

In 1928 and 1929 Apperly and Semmons related plasma bicarbonate to gastric activity, and in 1931 with Crabtree, Apperly confirmed the observations of Dickson and Wilson (1924). Studying also the secretory response they concluded that unlike the motor power of the stomach, gastric acidity varies with the fasting plasma bicarbonate irrespective of the H-ion concentration.

The sugar level in the blood has also been suggested as a modifier of gastric function. In 1924 Bulatao and Carlson produced inhibition of hunger

contractions by experimental hyperglycemia. They augmented gastric tone and brought on tetany with insulin hypoglycemia. La Barre's (1931) findings substantiate those of Bulatao and Carlson and add strong evidence that gastric acidity is similarly affected, the mechanism being a vagal one. Recently Regan (1933) has found the primary effect of insulin depressing, gastric motility being initiated only when the sugar fall is great enough for central parasympathetic stimulation. Quigley and Hallaran (1932) and Mulinos (1933) deny any direct relationship between the glucose level of the blood and gastric activity.

Violent and exhaustive exercise produces blood changes similar to those believed in themselves to affect gastric function. Simultaneous determinations were therefore made of gastric acidity, blood sugar, plasma bicarbonate and blood pH under conditions of rest and exercise.

METHODS. Sixteen observations were made upon a healthy young adult woman accustomed to strenuous exercise and to gastric intubation. Confirmatory data were obtained upon another subject, who, during the course of a similar series of experiments gradually developed a total anacidity unassociated with disease. The influence of three types of exercise was studied, both when the activity preceded and followed the test meal. The exercises selected were classified as mild, violent and exhaustive and have already been described (Hellebrandt and Hoopes, 1934). The mild exercise was planned to stimulate the circulation but leave the blood constituents essentially unchanged; the violent, to bring about variations in the acid-base balance of the blood; the exhaustive, to deplete, if possible, the reserves of carbohydrate.

The technique for the administration of the test meal and recovery of the gastric specimens is that described in the first paper (Hellebrandt and Hoopes, 1934). Blood samples were withdrawn under oil from the cubital veins. Ten to fifteen cubic centimeters were collected immediately before exercise, at its termination and one hour later when recovery had occurred. Plasma carbon dioxide capacity was determined by the method of Van Slyke and Neill (1924), plasma pH by the method of Cullen and Biilmann (1925), and blood sugar by the method of Folin and Wu (1919, 1920).

RESULTS. A typical protocol follows:

Exhaustive exercise preceding the meal.

12:05 Pre-exercise blood sample taken.

12:06 Exercise commenced. 12:06–12:26 pedalling the Kelso electrodynamic brake bicycle ergometer at 984 kilogram. m./min. 12:16 sweating profusely on brow and back. Face flushed. 12:26–12:41 working in the rowing machine at 36 pulls/min. Flushing continues. Arms blotched and red. 12:41–12:56 walking in the treadmill at a steep grade, 68 steps/min. Sweating freely. Dark circles under the eyes. 12:56–1:06 rotating the Prony brake hand ergometer, 40 turns/min. Cheeks brilliantly flushed. Subject dyspneic and greatly fatigued.

1:06 Cessation of exercise. Fifteen cubic centimeters blood withdrawn by venae puncture.
1:07 Rehfuss tube swallowed.
1:08 Fasting gastric contents aspirated. Twenty-seven cubic centimeters of cloudy colorless juice obtained with a free HCl of 10 and a total acidity of 30. Starch test negative.
1:10 Five hundred cubic centimeters warm oatmeal gruel introduced through the tube under gentle pressure.

		Free HCl	Total Acidity	Starch
1:25	Sample 1 aspirated	0	10	+
1:40	Sample 2 aspirated	0	10	+
1:55	Sample 3 aspirated	0	6	+
2:06	Last blood sample withdrawn			
2:10	Sample 4 aspirated	0	14	+
2:25	Sample 5 aspirated	6	14	+
2:40	Sample 6 aspirated	12	26	+
2:55	Sample 7 aspirated	52	58	+
3:10	Sample 8 aspirated	28	48	?
3:25	Sample 9 aspirated	18	36	−

Emptying time 2 hours and 15 minutes; 55 cc. saliva aspirated.
Blood sugar: 0.928, 1.34, 0.90 mgm. per cent.
Carbon dioxide combining power: 57, 48.5, 61.5 volumes per cent.

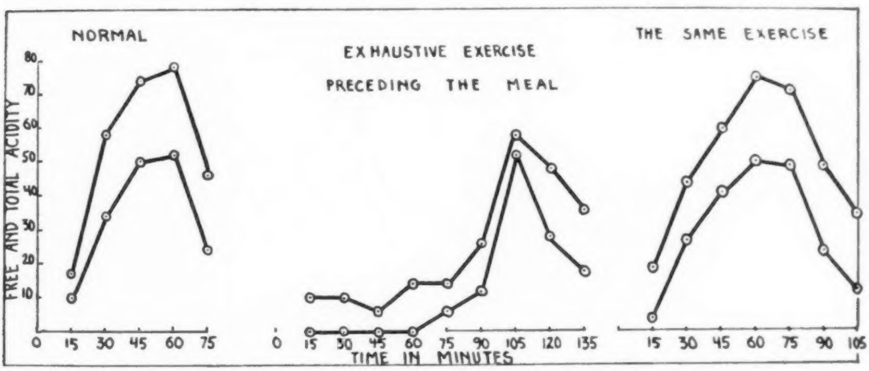
Brief violent exercise carried on at high speed was associated with the greatest reduction in carbon dioxide combining power. The average fall was 39 per cent of the original level. The greatest change observed was a drop from a pre-exercise value of 53.1 to a post-exercise one of 28 volumes per cent. Protracted, exhaustive exercise was accompanied by an average fall in carbon dioxide combining power of 26 per cent. One hour after the termination of both types of exercise the plasma bicarbonate had returned approximately to the pre-exercise value. Under the influence of mild exercise it remained essentially unchanged.

Violent exercise was associated with an increase in hydrogen-ion concentration, the average pH change being a fall from 7.51 to 7.46. The variation was in the same direction when the exercise was exhaustive, but mild activity produced no change.

In no instance was the exercise severe enough to affect the blood sugar level in the direction of a fall below the pre-exercise value. Instead, both types of severe muscular activity were associated with a concomitant increase in blood sugar. Short violent exercise elevated it by 23 per cent, exhausting, protracted muscular work by 27 per cent. It remained unchanged when mild activity comprised the exercise studied.

In spite of the fact that the subject upon whom the primary observations were made was a trained laboratory worker accustomed to swallowing the stomach tube, emotional stress occasioned by the experimental procedure was probably never completely eliminated. The violent and exhaustive exercises were of such severity that their performance gave no pleasure. They were carried on to the utmost of the subject's physical power and at their termination were always associated with distress and fatigue.

The experiments were conducted over a period of seven months, and as the exercises were repeated they had less and less effect upon gastric activity. The greatest variations in the secretory response occurred upon the first performances of the exercise procedures. This is well illustrated in graph 1, identical bouts of exercise measured in terms of their physical demands, producing a dissimilar effect less divergent from the normal as the subject became accustomed to the procedure. To counteract the effect of training the subject endeavoured to gradually increase the speed with which the various activities were performed, the duration of muscular work remaining constant. The greatest reduction in carbon dioxide combining power ever observed in this series of experiments occurred the last of five times when violent exercise was performed, and this was associated



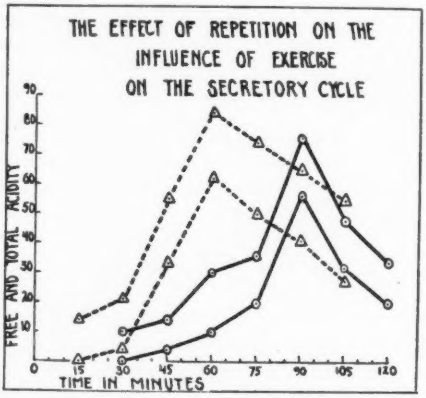
Graph 1. Acidity curves obtained on the same subject, showing the normal response and the effect of the first and fifth performance of exercise of comparable severity. The upper curves represent total acidity, the lower, free HCl.

with relatively slight change in the character of the gastric secretory cycle. This is illustrated in graph 2.

Discussion. Cannon (1909) had long since demonstrated the marked effect of the mental state upon the functions of the alimentary tract. Disagreeable emotional reactions may induce gastric stand-still. Steinhaus (1931a, b) in particular stresses the importance of the emotions among the causative agents contributing to the apparent effects of exercise upon gastric function. He also calls attention to the effects of training, citing the experiments of the Russian, Kadygrabow, whose Pavlov pouch dogs showed a diminishing secretory inhibition to exercise as the animals became accustomed to the muscular work they were required to perform. The experiments of Barcroft and Florey (1929) are particularly significant. They studied the effects of exercise on the turgidity of an exteriorized

patch of the mucous surface of the colon. Short bursts of running resulted in pallor which became less and less marked upon repetition of the exercise. They regard the pallor due in part to excitement, loss of interest in the exercise being associated with a diminution or a disappearance of the response. The colon exhibited only slight vaso-constriction and blanching during exercise of long duration.

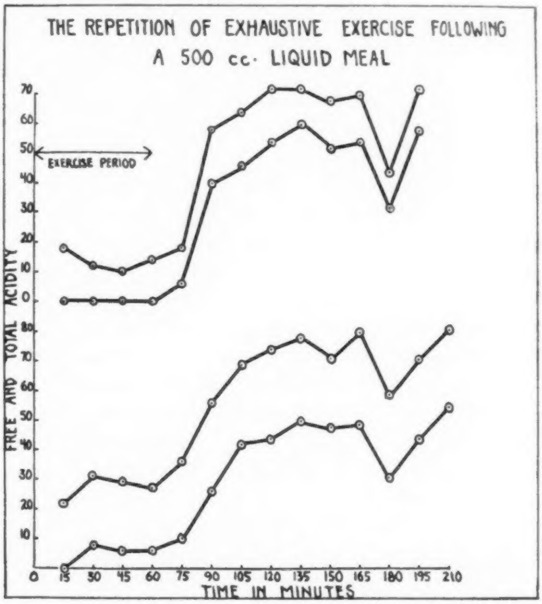
The violent and exhaustive exercises of our experiments were probably associated with a similar reflex vasomotor change, more marked when the procedure was novel than when it had been oft repeated. The reciprocal distribution of blood favoring the vessels of the active muscles during exer-



Graph 2. The solid lines represent the acidity response of one subject to the second performance of 3 minutes of violent exercise preceding the ingestion of a gruel meal. The plasma carbon dioxide combining power was 58.5 volumes per cent just before the exercise, 35 volumes per cent immediately after and 56 volumes per cent one hour later. The broken lines represent the response to the fifth performance of the same exercise. The corresponding carbon dioxide combining power values in volumes per cent were 53.1, 28 and 53. The upper lines indicate total acidity, the lower, free HCl.

cise has long been accepted. Activity as severe as that composing our strenuous exercise, must have been associated with a drainage of all available blood away from the visceral area. Splanchnic vasoconstriction throughout the protracted exercise must have subjected the stomach to anoxemia. Van Liere and Crisler (1930) have shown the inhibiting effect of anoxemia upon the hunger contractions of the dog. They have also demonstrated its depressing influence upon the digestive movements (Crisler, Van Liere and Booher, 1932). They believe the most plausible mechanism for the inhibition is a sensitization of the sympathetics to impulses normally subminimal, the agent being the rise in blood pH which comes on early in

anoxemia. Return to normal in their experiments on digestive contractions was prompt and complete immediately after the re-establishment of natural oxygen tension. However, a fall in blood pH occurred in every case of exercise hypoacidity which we induced by exercise. Anacidity of relatively long duration was also observed to occur even when the exercise preceded the meal. As is shown in graph 1, seventy-five minutes elapsed after a one hour battery of exhaustive exercise before any free HCl was detected in the aspirated gastric samples. Graph 2 shows a 45 minute



Graph 3. The top curve was obtained early in the period of experimentation, the bottom, after approximately six months of experience with the general procedure. The upper lines of each represent total acidity, the lower, free HCl.

delay following only 3 minutes of violent exercise. However, such rigorous muscular activity is associated with profound circulatory adjustments and post-exercise cardiovascular recovery is frequently very slow. The visceral anoxemia might persist long after the cessation of exercise.

When protracted exercise followed the ingestion of the meal, the acidity remained practically unchanged until the activity ceased. The rise was then prompt, as illustrated in graph 3. The physiological variability of the gastric secretory response is relatively great. The high degree of similarity in the two curves reproduced in graph 3 is therefore of particular

interest. It is to be noted that repetition of this type of exercise was not associated with a return of the acidity response to normal. Exercise of this severity is especially difficult to perform immediately after the ingestion of a large liquid meal, and repetition cannot alleviate the physical fullness and discomfort which 500 cubic centimeters of oatmeal gruel produce.

The secretory curves pictured in graph 2 were both obtained after a three minute bout of violent exercise. Immediately following the activity the carbon dioxide combining power had dropped from 58.5 to 35 volumes per cent in one case, and from 53.1 to 28 in the other. This determination was made upon a blood sample withdrawn before the aspiration of the first specimen of gastric juice. One hour later recovery was essentially complete, the carbon dioxide combining power having returned to 56 and 53 volumes per cent respectively. However, in the face of a normal plasma bicarbonate, the gastric juice contained in one instance a titratable acidity far below the normal level.

The data obtained from this limited series of observations point toward independence of blood changes and the composition of the gastric juice. Secretion is an active process involving the expenditure of energy. If one is justified in regarding gastric glandular function as analogous to salivary function, the hypothesis regarding the independence of blood changes and secretory function becomes more plausible. It has been shown that stimulation of the chorda tympani produces a different salivary fluid from that produced by stimulation of the sympathetic nerve, and this in spite of the fact that no blood changes have intervened. In his reviews Babkin (1927, 1928) makes no reference to the possible relation of blood changes to the composition of the gastric juice. Ivy (1925, 1927) stresses nervous and humoral mechanisms in his extensive observations on factors influencing gastric secretion, although he remarks that the "digestive blood state" may play some rôle. It seems especially "unphysiological" to ascribe a different mechanism to two phases of the work of a single organ as intimately related as the secretory and motor conduct of the stomach (Hellebrandt and Dimmitt, 1934).

SUMMARY

Exercise induces blood states identical with those resulting from other experimental manipulations known to be associated in themselves with similar gastric functional changes. When produced by exercise the influence of these physicochemical blood changes upon the composition of the digestive juice and the vigor of the response of the gastric musculature is no longer invariable. In the exercising human being some mechanism other than one related to the resultant changes in the blood must be responsible for the alterations in gastric function. It seems to us that in

the presence of the emotional stress and profound vascular readjustments which occur during strenuous exercise, physicochemical variations in the blood are concomitant rather than casual in their relation to alterations in gastric function.

We wish to thank the young women who kindly served as subjects in the series of experiments reported, and Dr. Carol Rice for her handling of the blood samples.

REFERENCES

Apperly, F. L. and M. G. Crabtree. 1931. Journ. Physiol., **73**, 331.
Apperly, F. L. and K. M. Semmons. 1928. Med. Journ. Australia, **2**, 226.
1929. Med. Journ. Australia, **2**, 192.
Babkin, B. P. 1927. Can. Med. Journ., **17**, 36.
1928. Physiol. Rev., **8**, 365.
Barcroft, J. and H. Florey. 1929. Journ. Physiol., **68**, 181.
Boldyreff, W. 1915. Quart. Journ. Exp. Physiol., **8**, 1.
Bulatao, E. and A. J. Carlson. 1924. This Journal, **69**, 107.
Cannon, W. B. 1909. Amer. Journ. Med. Sci., **137**, 480.
Crisler, G. E., E. J. Van Liere and W. T. Booher. 1932. This Journal, **102**, 629.
Cullen, G. E. and E. Bülmann. 1925. Journ. Biol. Chem., **64**, 727.
Dickson, W. H. and M. J. Wilson. 1924. Journ. Pharm. Exp. Therap., **24**, 33.
Folin, O. and H. Wu. 1919. Journ. Biol. Chem., **38**, 80.
1920. Journ. Biol. Chem., **41**, 367.
Hellebrandt, F. A. and S. L. Hoopes. 1934. This Journal, **107**, 348.
Hellebrandt, F. A. and R. H. Tepper. 1934. This Journal, **107**, 355.
Hellebrandt, F. A. and L. L. Dimmitt. 1934. This Journal, **107**, 364.
Ivy, A. C. 1925. Journ. Amer. Med. Assn., **85**, 877.
1927. Amer. Journ. Med. Sci., **173**, 453.
La Barre, J. 1931. Bull. Acad. Roy. Med. Belgique, **11**, 598.
Mulinos, M. G. 1933. This Journal, **104**, 371.
Quigley, J. P. and W. R. Halloran. 1932. This Journal, **100**, 102.
Regan, J. F. 1933. This Journal, **104**, 90.
Stewart, J. F. and W. N. Boldyreff. 1932. This Journal, **102**, 276.
Steinhaus, A. H. 1931. Journ. Health and Phys. Ed., **2**, no. 1, 40.
1931. Journ. Health and Phys. Ed., **2**, no. 2, 42.
Van Liere, E. J. and G. Crisler. 1930. This Journal, **93**, 267.
Van Slyke, D. D. and J. M. Neill. 1924. Journ. Biol. Chem., **61**, 551.

THE ACID-BASE COMPOSITION OF HEPATIC BILE: I

JOHN G. REINHOLD[1] AND D. WRIGHT WILSON

From the Biochemical Laboratory of the Philadelphia General Hospital, and the Department of Physiological Chemistry, School of Medicine, University of Pennsylvania, Philadelphia

Received for publication September 25, 1933

The digestive glands secrete fluids having characteristic compositions especially in regard to the enzymes and other organic constituents. In regard to the content of inorganic ions certain interesting relationships are shown. In the dog, for example, the parotid saliva obtained by stimulation with pilocarpin contains less sodium and chloride than the serum and more potassium, calcium and bicarbonate (deBeer and Wilson, 1932). Gastric juice from the active stomach is far more acid than serum and contains much more chloride but considerably less base (Gamble and McIver, 1928). Pancreatic juice is more alkaline than serum and contains more bicarbonate and potassium and less chloride and calcium (Ball, 1930). It is apparent therefore that each gland exerts considerable control over the composition of the fluid it secretes.

The comparatively large daily volume and the high content of solids in bile makes it a factor of some importance both as a secretory and excretory medium. Bile contains far more organic material than the other digestive fluids. In the dog, the organic substance is chiefly taurocholic acid and this fortunately can be determined quantitatively. Since studies of the acid-base relationships of the external secretions of other digestive glands have yielded information of considerable interest, it was decided to apply this approach to an investigation of hepatic bile obtained during periods of active secretion. Analyses of the principal ions, including the cholic acid, have been made and are to be reported in this paper. Other constituents of bile such as phospholipid and bile pigments have also been studied to some extent, although they appear to be of little importance in respect to acid-base relationships.

METHODS. In studying the composition of bile we have utilised both acute and recovery experiments on dogs. In the former, with the exception of a few animals, simple common duct fistulae were prepared under amytal anesthesia. The cystic duct was ligated and into the common duct

[1] This paper together with the two following has been presented by John G. Reinhold to the Graduate School of the University of Pennsylvania as a thesis in partial fulfillment of the requirements of the degree of Doctor of Philosophy.

there was inserted a glass cannula carrying about 10 cm. of rubber tubing. The fistula employed in the recovered animals was of the type described by McMaster and Elman (1925) permitting continuous flow of bile into the intestine by way of the common duct except when collection was actually in progress. In these animals also the cystic duct was ligated. The recovered animals were prepared under ether anesthesia by Dr. I. S. Ravdin of the Department of Surgical Research of the University of Pennsylvania Medical School, and the writers wish to express their appreciation for his assistance.

The dogs were maintained at approximately constant weight on a diet of cooked meat, bread and milk. Bile was collected in tonometers over mercury and handled so as to minimise losses of carbon dioxide and oxidation of the pigment. Blood was taken from the jugular veins in the acute experiments and from the saphenous veins in the recovery experiments. Cholic acid was determined according to the modification of the Gregory and Pascoe method described by Reinhold and Wilson (1932). It is important to note that both free and conjugated cholic acids respond to the Pettenkofer reaction under the conditions established. Desoxycholic and probably other naturally occurring bile acids give little or no color. Although most of the bile acid of dog bile is taurocholic acid, the method is not specific for this acid. Consequently the general term "cholate" is used in the tables and in most of the discussion. Chloride was determined by the method of Van Slyke as modified by Wilson and Ball (1928), and carbon dioxide by the constant pressure or constant volume apparatus of Van Slyke and Stadie (1921) and Van Slyke and Neill (1924). Correction has been made for free carbon dioxide according to the equation given by Peters and Van Slyke (1931) when pH figures were available. In the absence of pH determinations, an average correction of 1.5 milli-equivalents was subtracted from the total carbon dioxide. The results are recorded as bicarbonate.

Total solids were determined by drying at 105°C. to constant weight. Ashing was done in platinum dishes in an electric furnace at temperatures not exceeding 550°C. As bile proved to be resistant to simple ashing procedures, one or more extractions with normal hydrochloric acid and water were made after charring or partial ashing. Sodium was determined by the method of Barber and Kolthoff (1928) after removal of the phosphates by barium hydroxide. The titrimetric method of Shohl and Bennett (1928) was used for the determination of potassium, except that the chloroplatinate was usually centrifuged, instead of being separated by micro-filtration. Calcium was determined by the method of Clark and Collip (1925), and magnesium by the method of Briggs (1924). The method of Fiske and Subbarow (1925) was employed for phosphorus determinations. Phospholipid was calculated from the ether soluble phos-

phorus after digestion with nitric and sulfuric acids. pH determinations were made by the quinhydrone titrimetric method of Meeker and Reinhold (1928).

Bilirubin was estimated by direct comparison of the diluted bile, which had been kept from oxygen, with 0.025 per cent potassium bichromate. The latter was standardized by comparison with known solutions of purified bilirubin. Bichromate solutions of this strength proved to be equivalent to 0.38 mgm. bilirubin per 100 cc. and the results have been expressed in terms of the latter substance.

Post-mortem examination demonstrated that the ligatures on the cystic duct in every instance effectively eliminated the possibility of contamination of the hepatic bile with gall bladder bile. Atrophy of the gall bladder with thickening of the walls gave further proof that this organ was not functioning.

Experimental. A large number of experiments have been carried out. As all of the information obtained cannot be presented, data from characteristic experiments will be given in this and the following papers to illustrate the changes which occur as the result of the experimental procedure.

The analyses of a number of specimens of bile and serum collected at varying intervals after operation from six dogs are summarised in table 1.

It may be seen that the cholate[2] of dog bile constitutes a large proportion of the total anion, but that the concentration varies over wide limits. Lower values (13 to 56 milli-equivalents per liter) were observed in the acute experiments[3] probably as the result of exposure and manipulation of the liver. In the recovered animals the cholate concentration was lower during the first few days following the operation than it was subsequently when further recovery had occurred. Cholate was at a maximum concentration at the beginning of a day's experiment, and diminished as bile was withdrawn from the animal. In most experiments the rate of secretion of bile decreased at the same time, so that a connection appeared to exist between the rate of secretion and cholate concentration. Many conspicuous exceptions were encountered, however, and the fact that the highest observed concentrations of cholate were associated with unusually sluggish secretion of bile makes it obvious that other factors at times may upset this relationship.

[2] It should be remembered that the cholate of dog bile is present almost entirely as taurocholate. The latter acid ranks among the stronger organic acids (Hammarsten, 1924; Henriques, 1930; Josephson, 1933). Therefore it must be present entirely as a salt at the pH of bile.

[3] Results of acute experiments are not summarised in table 1. Examples may be found in tables 3 and 4 of the second paper and tables 1 and 2 of the third paper of this series. The data obtained from the fore periods of each experiment may be compared with the data in table 1 of this paper.

TABLE 1

Composition of hepatic bile and serum of six dogs

DOG	DAYS AFTER OPERATION	HOUR OF COLLECTION	RATE	SOLIDS	BILI-RUBIN	ETHER SOL. P	FREE CO_2	HCO_3	Cl	CHO-LATE	Na	K	Ca	Mg	UNDE-TER-MINED ANION	pH
			cc./min.	per cent	milli-mols per liter		milli-equivalents per liter									
23*	2	1st	0.70	6.2	0.14			26	88	63	172	6.2	8.2	2.8	13	
		3rd	0.13	2.6	0.36			33	104	17	141	4.6	5.5	2.5	0	
	7	1st	0.34	5.6	0.25			33	80	75	175	6.5	6.5	2.2	1	
24	4	1st	0.29	6.7	0.35	23	1.3	39	70	64	177					7.57
		3rd	0.11	4.5	1.50		1.4	61	79	26	159					7.71
	6	1st	0.12	7.4	0.46		1.6	42	68	72	178					7.52
26	3	1st	0.52	6.1	0.28	18	1.5	39	71	64	171					7.50
		3rd	0.33	4.6	0.41		1.3	47	79	35	168					7.65
27	5	1st	0.08	12.2	1.53	42		33	31	125	203		13.8			
	7	1st	0.13	10.5	1.76	43		33	42	92	172	7.5	11.0	5.0	26	
	8	1st	0.40	9.4		21		28	42	94	164	8.8	10.6			
28	5	1 + 2	0.08	4.9	0.58		1.8	52	52	56			6.4			7.55
		3 + 4	0.06	4.3	0.68		1.4	60	59	45			6.6			7.74
30	3	1st	0.29	5.4			1.0	14	93	28	164	5.4	7.7	3.8	46	7.23
		3rd	0.27	4.4			1.1	20	93	23	150	4.8	6.0	3.2	29	7.34
	7	1st	0.40	6.1	0.31		1.1	22	76	71		5.6				7.39
	16	1st	0.14	5.7	0.90			35	76	48			6.6	4.2		
	21	1st	0.56	8.5				14	66	94			8.2	2.3		
		3rd	0.09	5.2				14	76	41			7.8	5.5		
Av.	2 to 21	1st	0.29	7.1	0.70		1.5	34	64	76	174	6.6	8.6	3.6	26	7.46
Serum Av.				9.0				22	110		148	5.3	5.5	1.9		7.40†

* Data in table 2 (first hour) have been included in the averages.

† Taken from the literature.

Besides the bile acids the principal anions of hepatic bile are chloride and bicarbonate. A close relationship was constantly observed between the concentrations of chloride and bicarbonate on the one hand and cholate on the other (table 2). The amounts of the inorganic[4] anions depended primarily upon the concentration of the cholate. When this was small the concentration of inorganic anions was increased sufficiently to replace a large proportion of the cholate, and vice versa. However, the inorganic anions never fully replaced the cholate when the concentration of the latter changed, and alterations of the total base concentration always accompanied changes in the concentration of the cholate.

TABLE 2

The composition of bile after diversion of flow from the intestine and after the injection of bile into the duodenum

Feb. 29, 1932. Dog 23. Female, 14 kgm., 5 days after operation. Fasted 15 hours.

TIME	VOLUME	RATE	SOLIDS	PIGMENT	HCO_3	Cl	CHOLATE	Na	K	Ca	Mg	UNDETERMINED ANION
	cc.	cc./min.	per cent	mM.	milli-equivalents per liter							
11:50–12:45	15.2	0.28	7.46	0.62	22.7	64.8	101.5	178.0	5.8	7.7	2.4	4
12:55–2:35	13.2	0.15	6.79	0.50	41.8	62.2	78.0	166	6.0	8.2	2.8	0
2:43–4:51	13.4	0.11	4.91	0.50	56.1	67.7	48.5	166	5.1	7.0	3.0	7
4:56–*6:40	10.3	0.09	4.31	0.54	55.7	69.0	37.8	157	5.0	7.2	2.8	3
7:01–8:20	8.6	0.10	6.09	0.56	50.0	66.1	55.3	162	5.5	7.9	3.2	6

* Between 5:10 and 6:40, 8.6 cc. of the dog's own bile were injected into the duodenum by way of the common bile duct.

Bicarbonate concentration ordinarily was influenced far more than chloride by variations of the cholate concentration, and a decrease in the cholate generally caused a simultaneous fall in the hydrogen ion concentration of the bile owing to the substitution of the anion of the weak acid (carbonic) for that of the stronger acid (taurocholic). The reaction of the bile was usually more alkaline in the acute experiments where lower cholate concentrations occurred.

Bilirubin, if it binds base at the pH of bile, would be of minor importance

[4] It is convenient to include bicarbonate with the inorganic ions in order to easily distinguish it from the complex organic cholate.

in the acid-base equilibrium owing to the low molar concentration. Inorganic phosphate concentration was likewise insignificant. Often none could be detected by sensitive methods. Wohlgemuth (1903) found only traces of inorganic phosphate and sulfate in dog bile.

As the sum of the anions was always less than the sum of the cations, the presence of undetermined anion is suggested. The difference averaged about ten percent of the total anion though wide variations occurred, irrespective of the pH. Shortly after operation in one animal the undetermined anion exceeded the cholate concentration. After a time, the latter increased while the undetermined anion decreased until a more normal relationship was established. The possibility should not be overlooked that desoxycholic acid or similar compounds not yielding appreciable color with the method used for determining bile acids may be present in the undetermined anion fraction.

Next to the conjugated cholic acid, the phospholipid[5] of dog bile is quantitatively the most important constituent. Assuming that all of the ether soluble phosphorus was in phospholipid of the approximate molecular weight of lecithin, this group of substances together with taurocholate and the inorganic salts accounted for a large proportion of the solid matter in many of the specimens. It is probable, however, that these phospholipids do not bind appreciable amounts of base in bile (Levene, Rolf, and Simms, 1923).

The cation of the bile is predominantly sodium. Consequently, variations in the sodium accounted for practically all of the significant alterations in the total base of the bile. Without exception, the cations of the bile equalled or exceeded their concentrations in the serum. These differences were at times considerable, and were most noticeable in the case of calcium, which occasionally reached a value more than twice that of serum. However, in the acute experiments, concentrations of calcium and other cations were more nearly equal in bile and serum. Von Beznak (1931) has reported approximately equal concentrations of calcium in the two fluids.

The highest sodium concentrations in bile were associated with the highest cholate concentrations. The variations of these two constituents are illustrated in table 2. The high concentrations of sodium were always higher than the concentrations of that ion in serum. Only when the cholate was low did the sodium concentration in bile approach that in serum. The total molar concentration of anions and cations in bile exceeds a similarly calculated value for serum, and the difference between bile and serum becomes greater the higher the concentration of cholate in bile. Nevertheless, other investigators have consistently found that the

[5] The writers wish to express their indebtedness to Mr. George R. Kingsley for carrying out the lipid phosphorus determinations.

osmotic pressure of the bile is approximately the same as that of serum (Tabulae Biologicae, 1925; Ravdin, Johnston, Riegel, and Wright, Jr., 1932; Gilman and Cowgill, 1933). Even gall-bladder bile has about the same freezing point depression as hepatic bile and serum in spite of the great increase in concentration of certain constituents. Ravdin and his co-workers called attention to the fact that the freezing point of bile can be approximately accounted for by the osmolar concentration of the base, chloride, and bicarbonate.

Assuming that the osmotic pressure of bile remains practically constant, it is surprising to encounter considerable variations of total base. Undoubtedly, many factors need to be considered in arriving at a correct understanding of the phenomenon. One explanation is that taurocholic acid, though a strong acid and therefore present in the bile as a salt, is nevertheless highly aggregated so that it shows little osmotic activity. According to this explanation, as taurocholic acid decreases in concentration in the bile, it is replaced by chloride and bicarbonate ions, but as these are osmotically far more active the total anion-cation concentration must decrease if the osmotic pressure is to remain the same.

H. Hammarsten (1924) has presented evidence to indicate that aggregation may not account for the properties of taurocholic acid. He has shown that the freezing point depressions of solutions of taurocholic acid and sodium taurocholate are less than those calculated by assuming complete dissociation. His studies led him to the conclusion that while aggregation and diminished dissociation may possibly play a rôle, the facts would suggest that some of the base is rendered osmotically inactive by the molecules of taurocholic acid.

The change in the composition of the bile during a collection period that extended over several hours is a factor of importance in experimental studies dealing with bile (table 2). Thus the rate of secretion fell progressively, and the concentrations of solids, base, and cholate decreased. Bicarbonate rose during this interval with an accompanying shift in pH toward the alkaline. The alterations in the bile over three hour periods in several experiments are summarised in table 1.

That a decline in bile solids and in rate of secretion are consequences of the diversion of the flow of bile from the intestine has been demonstrated by the early students of biliary secretion (Stadelmann, 1891, 1896). In our experiments the steadily decreasing concentration of cholate[6] explains not only the decline in solids but also the rise in bicarbonate and the shift in reaction toward the alkaline. With depletion of the readily mobilised supply of preformed bile acids by the removal of bile, continuing secretion of bile acids becomes dependent to an increasing extent on the

[6] Greene, Aldrich, and Rowntree (1928) have described the decrease in bile acid content of bile following the interruption of the entero-hepatic circulation.

synthesis of these substances. A lowered concentration in the bile may follow. A further consequence of interrupting the flow of bile into the intestine is the loss of the stimulus to bile secretion that is provided by absorption of bile acid from the intestine. Obviously, other factors will contribute to the changes in the composition of bile observed under these circumstances, although certain agencies that might have been important in this connection have been excluded in the present experiments. These include stasis, gall bladder activity, and with less certainty, the concentrating activity of the ducts. As all of the experiments were begun in the morning at approximately the same time, the possible contributing influence of diurnal cyclic activity of the liver has not been overlooked. Injection of bile into the duodenum reversed the usual sequence of changes in the bile as is shown in table 2, but did not fully restore the bile to its initial composition.

An animal with a partial fistula secreted bile of more uniform composition than did animals with complete fistulae. In this instance one of the hepatic ducts had unintentionally been allowed to remain outside of the fistula system. Cholate concentration remained practically unchanged during the usual collection periods while the rate of secretion diminished only moderately.

As a result of the characteristic decline in rate of secretion and cholate concentration that occurred ordinarily in the fistula animals, it was at times desirable to accelerate the output of bile. Chemical stimulation by bile salts generally resulted in a marked response that frequently overshadowed the more moderate alterations caused by other substances. We have found, as has Jacoby (1931) that stimulation by the application of heat over the liver area is a much more satisfactory method of obtaining mild choleresis. For this purpose the abdomen was warmed by a 60 watt electric lamp at a distance of three inches, with several layers of gauze as a protection for the skin. This procedure frequently caused the flow of bile to increase two to three fold within an hour. The response was not confined to the rate of secretion for in many instances the concentration of cholate in the bile increased as well. The explanation for these stimulating effects of locally applied heat may be supplied in part by the increased circulation of blood through the vessels supplying the liver (Schwiegk, 1932). This author suggests that the effect on the circulation is reflex in nature.

SUMMARY

The composition of the bile of dogs has been studied over periods of four to eight hours by means of acute and recovery experiments.

The concentration of each of the cations in bile was usually higher than the concentration of the corresponding cation in serum. The principal anions were cholate (taurocholate), chloride, and bicarbonate.

The bile acid was quantitatively the most important constituent of the bile, although the amount of phospholipid was nearly as great. The two accounted for all but a small portion of the total solids in many specimens. As the pH of bile is within the isoelectric range of the ether soluble phospholipid it need not be considered in relation to anion-cation variations.

Phosphate was found only in traces. Appreciable amounts of undetermined anion were usually present.

Although the sum of the molar concentrations of anions and cations in bile exceeds that in serum, the osmotic pressure of the two fluids, as shown by the work of others, is practically the same. Actually the molar concentration of the inorganic ions is approximately the same in both. It would appear therefore that the principal organic ion of dog bile, taurocholic acid, either exhibits little osmotic activity or diminishes the osmotic activity of other ions.

The bile acid concentration appears to be the dominant factor in the regulation of the acid-base composition of bile. A decrease in cholate concentration was balanced partly by a decrease in sodium and partly by an increase in bicarbonate and chloride. The pH increased as a consequence of the rise in bicarbonate. When the bile acid concentration increased the changes were reversed.

Diversion of the flow of bile from the intestine was followed by a decrease in the concentration of cholate that led to the other changes just described. The decrease in cholate was most evident in recently operated animals. An animal with a partial fistula did not exhibit these changes.

Application of heat over the liver area stimulated the flow of bile and caused the cholate concentration to rise.

REFERENCES

Ball, E. G. 1930. Journ. Biol. Chem., **86**, 433, 449.

Barber, H. H. and I. M. Kolthoff. 1928. Journ. Amer. Chem. Soc., **50**, 1625.

Briggs, A. P. 1924. Journ. Biol. Chem., **59**, 255.

Clark, E. P. and J. B. Collip. 1925. Journ. Biol. Chem., **63**, 461.

deBeer, E. C. and D. W. Wilson. 1932. Journ. Biol. Chem., **95**, 671.

Fiske, C. H. and Y. Subbarow. 1925. Journ. Biol. Chem., **66**, 375.

Gamble, J. L. and M. A. McIver. 1928. Journ. Exp. Med., **48**, 849.

Gilman, A. and G. Cowgill. 1933. This Journal, **104**, 476.

Greene, C. H., M. Aldrich and L. G. Rowntree. 1928. Journ. Biol. Chem., **80**, 753.

Hammarsten, H. 1924. Biochem. Zeitschr., **147**, 481.

Henriques, O. M. 1930. Acta Pathol. et Microbiol. Scand., Supplement iii, 141.

Josephson, B. A. 1933. Biochem. Zeitschr., **263**, 428.

Jacoby, H. 1931. Arch. exp. Path. u. Pharm., **161**, 64.

Levene, P. A., I. P. Rolf and R. Simms. 1923. Journ. Biol. Chem., **58**, 859.

McMaster, P. D. and R. Elman. 1925. Journ. Exp. Med., **41**, 513.

Meeker, G. H. and J. G. Reinhold. 1928. Journ. Biol. Chem., **77**, 505.

PETERS, J. P. AND D. D. VAN SLYKE. 1931. Quantitative clinical chemistry, vol. i, Baltimore.
RAVDIN, I. S., C. G. JOHNSTON, C. RIEGEL AND S. L. WRIGHT, JR. 1932. This Journal, **100**, 317.
REINHOLD, J. G. AND D. W. WILSON. 1932. Journ. Biol. Chem., **96**, 637.
SCHWIEGK, H. 1932. Arch. exp. Path. u. Pharm., **168**, 693.
SHOHL, A. T. AND H. B. BENNETT. 1928. Journ. Biol. Chem., **78**, 643.
STADELMANN, E. 1891. Der Icterus und seiner verschiedenen Vormen. Nebst Beitragen zur Physiol. und Pathol. der Gallensekretion. Stuttgart.
1896. Zeitschr. Biol., **34**, 1.
Tabulae Biologicae. 1925. Volume ii, 508–9. Berlin.
VAN SLYKE, D. D. AND W. C. STADIE. 1921. Journ. Biol. Chem., **49**, 1.
VAN SLYKE, D. D. AND J. M. NEILL. 1924. Journ. Biol. Chem., **61**, 523.
VON BEZNAK, A. 1931. Pflüger's Arch., **228**, 604.
WILSON, D. W. AND E. G. BALL. 1928. Journ. Biol. Chem., **79**, 221.
WOHLGEMUTH, J. 1903. Cited by Kahn, M. and F. G. Goodridge (1926) in Sulfur Metabolism. New York and Philadelphia.

THE ACID-BASE COMPOSITION OF HEPATIC BILE

II. The Changes Induced by the Injection of Hydrochloric Acid and Inorganic Salts

JOHN G. REINHOLD and D. WRIGHT WILSON

From the Biochemical Laboratory of the Philadelphia General Hospital, and the Department of Physiological Chemistry, School of Medicine, University of Pennsylvania, Philadelphia

Received for publication September 25, 1933

Various glands have been found to differ in their permeability to intravenously injected inorganic ions as measured by the change in composition of their external secretions. Thus the pancreas is freely permeable to sodium, potassium, and bicarbonate, but less so to calcium, magnesium, chloride and phosphorus (Ball, 1930). The salivary glands permit the passage of the ions of calcium chloride and sodium carbonate into the saliva with greater readiness than is the case with certain other salts (DeBeer and Wilson, 1932). Only a limited amount of data is available concerning the effect of inorganic salts on the composition of the bile, and much of this has proved to be conflicting and inconclusive largely because of the complications resulting from operative procedures and the use of anesthetics, as well as the frequent lack of suitable controls. Few workers have considered bile acid concentration in connection with the studies of inorganic salt injections, although bile acid, as shown in the preceding paper, is a factor of fundamental importance in the regulation of the inorganic composition of the bile. Consequently a new investigation of this subject has been made, and in this paper there are reported experiments dealing with the effects of the injection of hydrochloric acid, sodium carbonate, and the chlorides of sodium, potassium, calcium, and magnesium on the composition of the bile. An attempt has been made also to relate the changes in this secretion to alterations in the composition of the serum caused by the injections.

The experimental and analytical procedures employed have been described in the preceding paper. Injections in the acute experiments were made slowly into the jugular vein, and in the experiments on the recovered animals into the saphenous veins. The solutions were warmed and precautions were taken to maintain the temperature of the animal at or near normal. Stimulation of the flow of bile by the application of heat over the liver area was employed in the acute experiments with few exceptions. To

conserve space, only one experiment is reported for each of the salts studied and the control experiments for each animal are not included. Tables 1 and 2 of the preceding paper provide examples of the characteristic changes that occurred in the control experiments. Results obtained from acute experiments have been verified in most instances by a repetition of the experiments upon recovered animals.

TABLE 1

The composition of bile after the intravenous injection of hydrochloric acid

May 12, 1932. Dog 26. Female, shepherd. Weight 20.5 kgm. 4 days after operation.

TIME	VOLUME	RATE	SOLIDS	PIGMENT	FREE CO_2	HCO_3	Cl	CHOLATE	Na	UNDETERMINED ANION†	pH
	cc.	*cc./min.*	*per cent*	*mM./liter*	*milli-equivalents per liter*						
9:32–9:57	10.7	0.43	7.09	0.33	1.4	27.1	68.2	85.3	171	4	7.40
10:00–10:42	13.2	0.33	6.86	0.49	1.3	24.2	69.5	73.8	171	16	7.37
10:45–11:09 *	8.0	0.33	6.20	0.53	1.3	28.5	74.6	58.8	171	22	7.43
11:10–11:30	8.3	0.42	5.21	0.40	1.3	34.6	79.0	45.9	159	13	7.52
11:30–12:10	8.6	0.23	4.09	0.57	1.5	41.0	81.8	29.8	152	11	7.54
12:10–1:20	10.7	0.15	2.98	0.75	1.4	39.2	83.8	17.8	146	17	7.54

* Ten cubic centimeters 1 normal hydrochloric acid injected into the left saphenous vein.

† Undetermined anion was calculated assuming the concentrations of potassium, calcium, and magnesium found in the control experiments.

EXPERIMENTAL. *Injection of hydrochloric acid.* The "paradoxical response" of the bile to the injection of hydrochloric acid reported by Hug and Marenzi (1928) seemed sufficiently remarkable to suggest further search for the cause. Instead of becoming more acid after the injection of hydrochloric acid, these workers observed that dog bile became considerably more alkaline, a result that led to the conclusion that the acid-base regulation of the bile is dissociated from that of the blood. The work of Arakawa (1927) is not in accord with such a conclusion for he reported that the pH of the bile of rabbits varied with the pH of the blood.

In our experiments the pH of the bile rose following the injection of 0.3 N hydrochloric acid while at the same time the pH of the serum fell, confirming the observations of Hug and Marenzi. Only after an unusually large injection of hydrochloric acid (190 cc. of 0.3 N) did the bile pH and serum pH diminish together. In all experiments, chloride concentration and rate of secretion increased following the injection. The acid was usually administered in 0.9 per cent sodium chloride solution but the effect of the latter would be negligible in the quantities given. The increased pH of the bile was shown by further experiments on two recovered animals to be related to the effect of the injected acid on the cholate concentration. Intravenous administration of hydrochloric acid was followed by a fall in the concentration of the cholate (table 1). Total base declined, but not in proportion to the decline in the cholate. The resulting deficit of anion was compensated partly by a rise in chloride and partly by an increase in bicarbonate. The gain in the latter was sufficient to explain the shift in the reaction of the bile toward the alkaline.

This tendency toward an inverse change in bicarbonate when the cholate rose or fell has been pointed out in the previous paper. These experiments on hydrochloric acid injection, resulting in a change in pH opposite to that which might be expected, provide additional evidence to indicate that the variations in pH of bile are secondary to alterations in the concentrations of cholate and bicarbonate.

Injection of sodium carbonate. Sodium and bicarbonate concentrations in bile tended to rise after injections of sodium carbonate when the concentrations of both in the serum were increased (table 2). However, the concentration of bile acids and the rate of flow influenced the changes in these ions. Owing to the usual decline of the cholate concentration in the course of the collection of a series of samples of bile and the accompanying substitution of bicarbonate, it was difficult to demonstrate that excretion of bicarbonate resulted from the injection.

The rate of secretion was approximately doubled for short periods after the sodium carbonate injection in two of the three recovered animals that were tested. It is noteworthy that in the dog that did not respond with increased flow of bile, the rise in the concentration of injected ions was far greater than in the animals that responded with choleresis.

In the experiments that have been described and in an acute experiment in which sodium bicarbonate was given intravenously, there was no significant change in the hydrogen ion concentration of the bile. This is in agreement with the work of Ottenberg and Kahn (1932) who found that bile pH remained constant after oral or intravenous administration of sodium bicarbonate to dogs. Carnot and Gruzewska (1926, 1927) on the contrary reported marked changes in the pH and bicarbonate of the bile of dogs after the injection of large quantities of sodium bicarbonate.

Stransky (1931), who injected rabbits with sodium bicarbonate, also found that the bile bicarbonate rose. Moderate gains in sodium and bicarbonate concentrations have been reported also by Beckmann (1928) after sodium bicarbonate injection.

TABLE 2

Composition of bile following the injection of sodium carbonate

March 22, 1932. Dog 24. Female collie. Weight 13.6 kgm. 6 days after operation.

TIME	VOLUME	RATE	SOLIDS	PIGMENT	FREE CO_2	HCO_3	Cl	CHOLATE	SODIUM	pH
					Bile					
	cc.	*cc./min.*	*per cent*	*milli-mols per liter*	*milli-equivalents per liter*					
10:00–11:05	7.6	0.12	7.41	0.46	1.6	40.7	68.4	72.2	178	7.52
11:05–12:37	9.9	0.10	7.21	0.42	1.7	39.0	70.0	63.5	176	7.55
12:25–12:36*										
12:37–1:08	7.0	0.23	7.34	0.39	1.6	41.2	68.5	62.4	179	7.52
1:10–2:25	7.2	0.10	6.88	0.77	1.7	47.7	70.4	50.6	172	7.56
2:25–4:39	6.1	0.04	6.87	1.35	1.9	50.7	70.0	43.0	173	7.53
					Serum					
12:03			8.01			18.2	114.1		152	
12:45			7.25			30.2	112.9		160	
4:05			7.85			26.5	115.3		154	

* Two and eight-tenths grams sodium carbonate injected as 10 per cent solution into saphenous vein.

Injection of NaCl. Most of the previous work on the effect of NaCl injections on the bile appears to indicate that it causes little change in this secretion. Adachi (1923) has found that the bile flow is stimulated by sodium chloride, while Faludi (1928) observed no effect on the rate. A slight increase in the concentration of sodium followed the administration of NaCl intravenously in the experiments of Beckmann (1928), but not until 60 minutes after the injection. Chloride decreased steadily during

his experiment and there was a decided diminution of the rate of flow. Ohta (1930) reports the absence of change in sodium and chloride of the bile following sodium chloride injection, and the work of Stransky (1931) on rabbits leads to the same conclusion.

TABLE 3

The composition of bile after the intravenous injection of sodium chloride

August 30, 1931. Dog 17. Male. Weight 14.2 kgm. Acute experiment, amytal anesthesia.

TIME	VOLUME	RATE	SOLIDS	HCO_3	Cl	CHOLATE	Na	UNDETERMINED ANION†
				Bile				
	cc.	*cc./min.*	*per cent*	*milli-equivalents per liter*				
11:05–12:20	6.4	0.09	2.87	68.5	81.1	14.6	165	11
12:28–2:30	8.8	0.07	2.51	68.1	79.9	12.4	161	10
2:23–3:23*								
2:31–3:58	6.5	0.08	3.36	68.9	100.3	4.8	190	27
4:00–5:11	4.0	0.06	3.63	71.3	103.5	4.8	196	26
5:16–6:26	4.8	0.07	3.33	78.5	96.0	3.9	195	27
				Serum				
12:00			8.07	25.7	110.4		144	
1:55			8.23	25.3	111.2		144	
3:14			6.28	25.9	158.1		175	
4:35			7.48	24.7	145.4		171	
6:17			7.87	24.3	143.1		167	

* Fourteen and two-tenths grams sodium chloride injected as 10 per cent solution into jugular vein.

† Undetermined anion was calculated assuming the concentrations of potassium, calcium, and magnesium found in the acute control experiments.

Preliminary trials indicated that the failure of the workers mentioned to obtain significant effects from NaCl injections was due to the relatively large quantities of this substance needed to bring about appreciable changes in the sodium and chloride content of the serum (table 3). One gram per

kilogram of body weight as ten per cent solution caused the sodium concentration of the bile to rise considerably in five experiments, while the administration of half this quantity led to a moderate elevation of the bile sodium in one experiment. Chloride and sodium in the bile increased together, although the rise in chloride usually exceeded the rise in sodium.

Cholate concentration diminished after the injection of sodium chloride. While usually loss of cholate ion was replaced by bicarbonate, chloride was substituted after sodium chloride had been administered. Bicarbonate decreased in about half of the experiments, and a comparable increase occurred in the hydrogen ion concentration. The cations other than sodium were determined in three experiments but no characteristic variations were observed.

The flow of bile was accelerated in most instances by the intravenous administration of large quantities of sodium chloride. This was frequently associated with strong diuresis, which suggested that the stimulus leading to the increased flow of bile was not confined to the liver. Hydremia, as shown by the diminished content of solids in the blood serum, may have been a factor. After sodium chloride, hydremia and choleresis were coincident, and the increased flow of bile terminated when serum solids returned to the preinjection levels. Faludi (1928) had previously been unable to correlate choleresis with the hydremia induced by injections of smaller quantities of sodium chloride. Sodium dehydrocholate choleresis greatly augmented the excretion of injected sodium chloride by way of the bile.

Injection of potassium chloride. The well-known toxic effects of intravenously injected potassium chloride led first to the use of a small amount of this salt for the injection. Little change in the bile potassium followed. Subsequently, trial injections demonstrated that comparatively large amounts of potassium chloride could be administered by way of the saphenous vein without danger to the animals. When a recovered dog was so injected, the experiment showed that a surplus of potassium in the serum is excreted partly by way of the bile. Both chloride and potassium concentrations rose to a somewhat greater extent in the bile than in the serum (table 4). The flow of bile also was accelerated for a short time after the injection.

Cholate concentration of the bile declined immediately after the injection, and, as usual, the decrease in cholate was accompanied by increased bicarbonate, with a resulting higher bile pH. pH and bicarbonate soon returned to their original values.

Injection of potassium chloride was followed by a slight gain in bile potassium in an experiment reported by Beckmann (1928) and chloride rose appreciably. Increased secretion of bile (Heianzan, 1925) and decreased secretion of cholic acid (Okamura, 1931) have been reported after injections of potassium chloride.

Injection of calcium chloride. Because of the small proportion of injected calcium chloride that appeared in the urine of dogs, Whelan (1925) examined the bile as a possible channel of excretion. Partly due to a rise in concentration and partly to increased flow of bile, the elimination of calcium was more than doubled. At the same time the output of chloride

TABLE 4

Effect of potassium chloride injection on the composition of bile and serum

July 15, 1932. Dog 30. Female, shepherd. Weight 22.0 kgm. 7 days after operation.

TIME	VOLUME	RATE	SOLIDS	PIGMENT	FREE CO_2	HCO_3	Cl	CHOLATE	K	pH
				Bile						
	cc.	*cc./min.*	*per cent*	*milli-mols per liter*	*milli-equivalents per liter*					
9:25–9:50	10.2	0.40	6.09	0.31	0.9	21.1	75.6	71.0	5.6	7.39
9:51–10:27	9.2	0.26	6.72	0.35	0.9	14.3	69.2	76.0	5.3	7.28
10:28–10:54	5.8	0.22	6.21	0.51	0.9	16.5	74.4	73.2	5.4	7.35
10:44–10:54*										
10:55–11:28	8.9	0.27	4.87	0.43	1.0	21.1	80.8	50.0	7.8	7.41
11:28–12:11	9.1	0.21	4.94	0.45	0.9	15.4	79.2	50.4	6.3	7.31
				Serum						
10:44			9.41				113.2		5.0	
11:03			10.29				114.0		6.2	

* Two and nine-tenths grams potassium chloride injected as 10 per cent solution into saphenous vein.

rose, although the concentration decreased. Dittrich (1924), Ipponsugi (1926), Guassardo (1929), Ohta (1930), Mirvish, Sacks, and Schrire (1930), and Beckmann (1928) found that the calcium concentration in the bile was higher after injection of calcium salts. Drury (1924) observed no effect on the 24 hour output of calcium in the bile after injections of calcium chloride intravenously. Inspection of the data presented in these

papers shows that exceptions are common, and furthermore, that there is no agreement concerning the importance of the bile as an excretory path for calcium.

The discrepancies may in part be related to the widely differing amounts of calcium administered by the various workers, for the present experiments clearly demonstrated that the response is dependent on the amount of calcium chloride administered. Smaller quantities increased the rate of secretion of bile, but had no effect on concentration of the injected ions in the bile. Injections of somewhat larger amounts of calcium chloride caused no change in rate of secretion but increased the concentration of calcium and chloride in the bile. Still greater quantities, sufficient to cause vomiting and convulsions, suppressed the flow of bile completely for a short period, while later the secretion was resumed at a slow rate. In the last instance, bile calcium and chloride were considerably increased. A like amount of calcium chloride administered to a dog under amytal anesthesia failed to exert a toxic action. In this experiment rate of secretion and concentration of calcium and chloride were simultaneously increased (table 5). Chloride always rose more than calcium. When calcium chloride was injected during periods of active choleresis induced by sodium dehydrocholate, the rise in concentration of chloride and calcium was relatively moderate, but owing to the rapid flow, excretion via the bile was greatly accelerated.

In the recovered animals calcium chloride exerted an inhibiting action on cholic acid secretion resembling the effect of hydrochloric acid, although not nearly so marked as the latter. A similar action is described by Okamura (1931). This effect was not observed in the acute experiment where, under amytal anesthesia, the cholate excretion increased after calcium chloride, although concentration of cholate decreased slightly.

A transitory fall in pH immediately following the injection in the recovered animals was rather unexpected, coming at the same time as a decline in cholate. Somewhat similar changes occurred after NaCl injections.

Injection of magnesium chloride. Laborde in 1879 reported that magnesium chloride stimulated the flow of bile despite the toxic action of the salt. An inhibitory action on bile flow by intravenous magnesium sulfate was observed by Chabrol and Maximin (1928) although the same authors reported subsequently (1929) that magnesium chloride (in half the quantity) stimulated the flow of bile. Increased magnesium and chloride in the bile after intravenous injection of magnesium chloride are described by Ohta (1930) and by Valdecasas (1931). The latter found simultaneous changes in blood and bile during the injection. However, owing to the spontaneous rise in the bile magnesium that frequently occurs in the course of the collection of a series of successive specimens, there is some

question as to whether the results of the latter authors may not represent such a spontaneous change in the magnesium concentration of the bile. The tendency of magnesium to increase during control collections contrasts with the simultaneous decrease in the concentrations of the remaining cations.

TABLE 5

The composition of bile after the intravenous injection of calcium chloride

September 11, 1931. Dog 18. Weight 26.5 kgm. Acute experiment, amytal anesthesia.

TIME	VOLUME	RATE	SOLIDS	HCO_3	Cl	CHOLATE	Na	Ca	UNDETERMINED ANION†
Bile									
	cc.	*cc./min.*	*per cent*	*milli-equivalents per liter*					
11:03–12:10	7.7	0.13	6.11	57.3	69.2	44.0	172	3.7	11
12:15–2:08	7.0	0.06	7.25	55.9	59.2	50.2	177	4.8	23
2:00–2:08*									
2:11–3:06	6.9	0.12	5.98	59.3	66.2	43.8	168	8.1	12
3:10–3:55	7.4	0.17	4.15	59.3	79.0	29.0	162	4.7	9
4:03–5:05	7.4	0.12	3.97	63.8	76.0	22.9	160	4.2	7
Serum									
11:40			9.30	23.1	112.8		149	3.2	
2:16			8.98	21.0	118.8		149	5.9	
3:35			9.65	20.6	116.8		141	3.8	

* Two and sixty-five-hundredths grams calcium chloride injected as 10 per cent solution into jugular vein.

† Undetermined anion was calculated assuming the concentrations of potassium and magnesium found in the acute control experiments.

Three experiments on recovered dogs were made before it was possible in the third experiment to clearly demonstrate a rise in the concentration of magnesium in the bile after injection of magnesium chloride (table 6). In other experiments the change in the bile was proportionately not any

greater than in the control experiments for the same animal although magnesium concentration in the serum gained five fold or more. In view of the marked elevation of serum magnesium that was needed to bring about excretion in the bile, it is evident that the latter is not an effective channel for the excretion of inorganic magnesium. However, bile chloride concentration increased in all of the experiments. Cholate declined moderately in two experiments but increased in a third. The flow of bile was accelerated moderately in two of three experiments.

TABLE 6

Effect of magnesium chloride injection on the composition of bile and serum

November 11, 1932. Dog 31. Female, shepherd. Weight 16.4 kgm. 50 days after operation. Partial fistula.

TIME	VOLUME	RATE	SOLIDS	HCO_3	Cl	CHOLATE	Ca	Mg
				Bile				
	cc.	*cc./min.*	*per cent*	*milli-equivalents per liter*				
10:40–12:14	6.2	0.07	6.93	2.9	105.2	59.1	7.7	2.2
12:15–1:55	9.0	0.09	7.24	2.9	104.0	62.1	8.0	2.5
1:51–2:06 *								
1:55–3:21	4.9	0.06	6.19	1.9	114.8	49.2	8.0	2.6
3:21–4:55	4.6	0.04	5.87	1.3	118.0	47.2	7.5	5.0
				Serum				
1:45					114.9		5.8	1.4
2:35					117.9		6.9	6.8

* One and sixty-five-hundredths grams magnesium chloride injected as 10 per cent solution into saphenous vein.

Discussion. In our studies when inorganic salts were injected in sufficient quantity to raise the concentration of the injected ions in the serum, the concentrations of these ions tended to increase in the bile. However, differences were observed in the facility of passage of the various ions from blood stream to bile. Of those that have been studied, chloride appeared to enter the bile most readily, magnesium the least so. Increased excretion of the injected material usually but not always followed intravenous administra-

tion. Excretion was promoted in many instances by more rapid secretion of the bile after the injection, although if the choleresis was pronounced, the rise of the concentration in the bile of the ions administered was restricted.

Owing to their action on the secretory mechanism of the liver, several of these substances caused changes in the composition of the bile that varied widely from those that might have been expected had the bile served simply as a pathway of excretion. The effect of intravenously injected hydrochloric acid on the liver provided a notable example. In this instance the secretion of bile acid was inhibited and the consequent increase in the bicarbonate concentration caused the bile to become more alkaline instead of more acid.

Choleresis was induced at times by each of the substances that have been injected. Hydrochloric acid, sodium chloride, and potassium chloride stimulated the secretion of bile in all experiments. Calcium and magnesium chlorides, and sodium carbonate inhibited the flow of bile in certain instances, although commonly these salts accelerated secretion. Other workers have also failed to obtain uniform effects on the rate of secretion of bile in experiments of this type. In the present experiments, the choleretic effect was most striking when the total ion concentration of the bile showed the greatest increase, although the association was by no means constant.

SUMMARY

Hydrochloric acid, sodium carbonate, and the chlorides of sodium, potassium, calcium and magnesium have been injected intravenously into bile fistula dogs and the composition of the bile has been studied before and after the injection.

The bile became more alkaline after the injection of hydrochloric acid. This may be attributed to a rise in the bicarbonate concentration that followed diminution of the concentration of cholate (taurocholate). Chloride concentration increased.

After injections of sodium carbonate the concentrations of the injected ions showed a tendency to increase in bile when they were increased in the serum. There was little change in the reaction of the bile.

Large quantities of sodium chloride had to be injected in order to bring about a significant change in the concentration of this substance in serum or bile. As chloride increased, cholate and bicarbonate decreased. The bile became more acid for a brief period. The augmented secretion of bile after the administration of sodium chloride appeared to be associated with the hydremia induced by the large quantities of salt.

Potassium chloride injections likewise caused the concentrations of the injected ions to rise in the bile. The cholate was lowered while, simultaneously, bicarbonate increased. The bile became more alkaline.

Intermediate quantities of calcium chloride stimulated the flow of bile without changing the concentrations of calcium or chloride. Larger injections, though causing marked systemic reactions which led to a diminished flow of bile, were followed by a considerable increase in the concentrations of calcium and chloride in the bile. When the toxic effects were suppressed by amytal, rate of secretion and concentrations of the injected ions were both increased.

The concentration of magnesium in the serum had to be raised considerably before a conclusive change in bile magnesium concentration occurred. The concentration of this ion in bile tended to increase during control collections.

The rate of secretion of bile was frequently accelerated by intravenous injections of hydrochloric acid and inorganic salts, while certain salts at times inhibited secretion.

REFERENCES

Arakawa, T. 1927. Ber. ges. Physiol. u. exp. Pharm., **42,** 689.
Adachi, A. 1923. Biochem. Zeitschr., **140,** 185.
Ball, E. G. 1930. Journ. Biol. Chem., **86,** 433, 449.
Beckmann, K. 1928. Deutsch. Arch. klin. Med., **160,** 63.
Carnot, P. and Z. Gruzewska. 1926. Compt. rend. Soc. biol., **94,** 756.
1927. Compt. rend. Soc. Biol., **96,** 912.
Chabrol, E. and Maximin. 1928. Bull. soc. méd. hop. Paris, **34,** 1693.
1929. Compt. rend. Soc. biol., **100,** 3.
de Beer, E. C. and D. W. Wilson. 1932. Journ. Biol. Chem., **95,** 671.
Dittrich, J. 1924. Zeitschr. ges. exp. Med., **41,** 355.
Drury, D. D. 1924. Journ. Exp. Med., **40,** 797.
Faludi, F. 1928. Zeitschr. ges. exp. Med., **61,** 121.
Guassardo, G. 1929. Pathologica, **21,** 180.
Heianzan, N. 1925. Biochem. Zeitschr., **165,** 33.
Hug, E. and A. D. Marenzi. 1928. Compt. rend. Soc. biol., **99,** 240.
Ipponsugi, T. 1926. Mitt. allg. Pathol. u. Pathol. Anat., **3,** 303.
Laborde. 1879. Cited by Chabrol and Maximin, 1929.
Mirvish, L., G. Sacks, and T. Schrire. 1930. Journ. Physiol., **70,** 434.
Ohta, R. 1930. Ber. ges. Physiol. u. exp. Pharm., **56,** 714.
Okamura, T. 1931. Chem. Abst., **25,** 4614.
Ottenberg, A. and J. H. Kahn. 1932. Proc. Soc. Exp. Biol. Med., **29,** 573.
Stransky, E. 1931. Zeitschr. ges. exp. Med., **77,** 807.
Valdecasas, J. G. 1931. Pflüger's Arch., **228,** 310.
Whelan, M. 1925. Journ. Biol. Chem., **63,** 585.

THE ACID-BASE COMPOSITION OF HEPATIC BILE

III. The Effects of the Administration of Sodium Taurocholate, Sodium Cholate and Sodium Dehydrocholate (Decholin)

JOHN G. REINHOLD and D. WRIGHT WILSON

From the Biochemical Laboratory of the Philadelphia General Hospital, and the Department of Physiological Chemistry, School of Medicine, University of Pennsylvania, Philadelphia

Received for publication September 25, 1933

The pronounced choleretic action of the bile acids has long been known and often investigated but detailed studies of the composition of bile following the administration of bile acids are few in number principally because of the lack, prior to the last few years, of satisfactory micromethods for their determination. The literature dealing with the effects of bile acid injections has been reviewed from time to time by Stadelmann (1891), Adler (1929), Jenke (1931–2), and Horsters (1932).[1] Considerable interest has recently arisen in the physiological and therapeutic properties of sodium dehydrocholate in this connection, because of its unusually powerful choleretic action. As a stimulant to bile flow, its action has been discussed by Faludi (1928), Wakefield, Powelson, and McVicar (1929), and Regan and Horrall (1932).

It has been shown in a preceding paper that the acid-base composition of the bile of the dog appeared to be governed primarily by the concentration of cholic acid (chiefly present as taurocholic acid). When this was diminished by the interruption of the enterohepatic circulation or by the injection of hydrochloric acid and certain inorganic salts, the total base of bile declined, while chloride and bicarbonate rose. Bicarbonate increased far more than chloride and as a consequence of the substitution of bicarbonate for cholate, the bile became more alkaline. If these changes are the result of the alteration in the concentration of the cholate, injection of bile acid should be followed by changes that are the opposite of those described. That this actually occurred is shown in the experiments that follow.

Experimental. Sodium taurocholate, sodium cholate and sodium dehydrocholate have been administered by injection into peripheral veins, peritoneum, and subcutaneous tissue and by infusion into the duodenum.

[1] Greene and Snell (1928) and Chabrol and Maximin (1928) have demonstrated that injected bile acids are transferred from blood stream to bile with great rapidity.

In general the response to the administration has been the same for the various paths of injection, differing mainly with respect to speed of action. Aside from hemolysis that resulted when sodium taurocholate was injected intravenously, the amounts of bile acids given were too small to exert appreciable untoward effects. The sodium cholate was prepared from cholic acid of proven purity, and purified commercial sodium taurocholate was used. Decholin Sodium, 20 per cent, (Riedel-de Haen) served as the source of sodium dehydrocholate. The experimental and analytic methods have been described in a preceding paper.

The effects of intravenous injection of sodium taurocholate are shown in the first part of table 1. A sharp increase in the concentration of cholate in the bile accompanied the augmented rate of secretion. There was a simultaneous rise in sodium concentration and a fall in bicarbonate. pH decreased presumably as a result of the displacement of the bicarbonate by the ions of the stronger taurocholic acid. The undetermined anion was reduced by half.

The marked rise in the concentration of sodium and cholate without a corresponding fall in chloride and bicarbonate presents a problem in regard to osmotic pressure calculation similar to that discussed in a previous paper. Assuming that the osmotic pressure of bile remains constant, it is obviously necessary to conclude that the osmotic pressure is not proportional to the total anion-cation concentration. Our former discussion mentioned the hypothesis of H. Hammarsten (1924) who believes that, while aggregation and lack of dissociation of the salt of taurocholic acid may play a part, it is necessary to assume that taurocholate exerts some further influence such as diminishing the osmotic activity of the inorganic cations.

The choleretic action of dehydrocholic acid was stronger than that of taurocholic acid, but the effect of the former was accomplished primarily by an augmented excretion of water. The contrast between its action and that of taurocholic acid is shown in table 1. Injections of 10 to 20 mgm. per kgm. caused the total solids of the bile to decrease considerably. Only when the quantity administered was very large, 100 mgm. per kgm., was the content of solids in the bile increased after the injection (table 2), and under these circumstances the cause was apparently the excretion of dehydrocholic acid. The cholate was lowered to nearly one-tenth of the preinjection level, and data have been obtained indicating that the phospholipid was also diminished, although not to the same extent. Neubauer's observation that bile solids are unchanged by dehydrocholate, cited by Kauftheil and Neubauer (1932), probably can be explained by the large amounts of this substance administered and its resultant excessive excretion. The dosage of sodium dehydrocholate used by these workers considerably exceeds that employed therapeutically in man.

TABLE 1

Composition of bile following the injection of sodium taurocholate and of sodium dehydrocholate

August 12, 1930. Male. Weight 13.8 kgm. Acute experiment. Amytal anesthesia

BILE	TIME	VOLUME	RATE	SOLIDS	BICARBONATE	CHLORIDE	CHOLATE	SODIUM	UNDETERMINED ANION†	pH
		cc.	*cc./min.*	*per cent*	*milli-equivalents per liter*					
1	11:30–2:23	7.3	0.05	4.5	71.2	52.5	21.1	160	27	7.56
	3:01*									
	3:26*									
2	2:56–3:45	9.8	0.20	7.40	49.1	53.6	82.1	187	12	7.41
	3:51*									
3	3:48–4:28	6.8	0.18	7.57	51.6	52.0	82.4	186	15	—
	4:35**									
4	4:36–5:05	3.6	0.25	4.95	50.8	57.5	22.4	161	31	—
	5:05**									
5	5:10–5:30	3.8	0.25	3.79	48.5	60.6	6.3	153	38	—
	5:30**									
6	5:30–5:58	6.1	0.32	3.57	43.4	63.5	9.4	149	33	—
	6:00**									
7	5:58–6:30	6.4	0.23	3.48	45.9	64.3	6.9	147	28	—

* Four-tenths gram sodium taurocholate injected intravenously.

** Two-tenths gram sodium dehydrocholate injected intravenously.

† Undetermined anion was calculated assuming the concentrations of potassium, calcium and magnesium found in the acute control experiments.

Perhaps the most striking change caused in the bile by sodium dehydrocholate injections was the almost complete replacement of the cholate by the undetermined anion. There is little doubt that the latter represents excreted dehydrocholate, since this substance does not respond to the color reaction used for cholate estimation. The increase in dehydrocholate (undetermined anion) concentration was accompanied by a considerable

TABLE 2

The effect of intravenous injection of sodium dehydrocholate on the composition of bile

June 3, 1930. Acute experiment. Dog 13. Male airedale. Weight 19 kgm. Amytal anesthesia.

TIME	VOLUME	RATE	SOLIDS	FREE CO_2	HCO_3	Cl	CHOLATE	SODIUM	UNDETERMINED ANION†	SODIUM DEHYDROCHOLATE EXCRETED‡	pH
	cc.	cc./min.	per cent	milli-equivalents per liter						mgm.	
1:10–3:13	11.8	0.02	3.22	2.8	82.4	54.3	13.1	162	25		7.60
3:10*											
3:13–3:30	13.0	0.77	3.75	2.0	37.8	62.6	2.8	153	61	199	7.38
3:30–3:56	15.0	0.59	4.02	2.0	34.4	62.2	1.8	150	63	242	7.34
3:56–4:33	13.6	0.37	3.74	1.3	41.2	61.0	4.5	146	51	150	7.59
4:33–5:10	8.5	0.23	3.57		49.2	64.2	7.4	150	41	58	
Total excretion										649	

* Two grams sodium dehydrocholate injected into jugular vein.

† Undetermined anion was calculated assuming the concentrations of potassium, calcium, and magnesium found in the acute control experiments.

‡ Sodium dehydrocholate = (milli-equivalents undetermined anion—milli-equivalents undetermined anion in preinjection specimen) × 0.424 × vol.

decrease in bicarbonate. The chloride concentration increased. Owing to the replacement of bicarbonate by dehydrocholate and chloride, the reaction of the bile became more acid.

The great increase in the undetermined anion that followed administration of sodium dehydrocholate suggested calculation of the quantity of this material that was excreted in the bile. If the gain in the undetermined

anion concentration over that present before the injection is expressed in terms of sodium dehydrocholate, such a calculation indicates that dog 13 (table 2) excreted about one-third of the injected dehydrocholate in two hours. Comparable figures have been obtained in other acute experiments, while similar calculations made for two experiments on a recovered dog indicated that approximately half of the dehydrocholate reappeared in the bile within two to four hours. The output actually may have been greater because of a decrease in the naturally occurring undetermined anion resulting from the tendency of injected bile acids to displace substances constituting this fraction. For example, taurocholate injections have been shown to cause a decrease in undetermined anion (table 1).

TABLE 3

Intravenous injection of sodium cholate

April 15, 1932. Dog 24. Female. Weight 13.7 kgm. 4 weeks after operation.

TIME	VOLUME	RATE	SOLIDS	HCO_3	Cl	CHOLATE	pH
	cc.	*cc./min.*	*per cent*	*milli-equiv./liter*			
10:40–1:55	4.9	0.03	7.47	38.1	69.2	68.8	7.12
1:57*							
2:40*							
1:57–3:50	4.0	0.04	7.76	23.4	62.0	76.5	6.97
3:52–5:42	3.2	0.03	6.24	30.1	66.4	64.0	—

* Two hundred milligrams sodium cholate injected subcutaneously.

As pointed out previously, the total molar concentration of ions in the bile always exceeded a similarly calculated total for serum, and the rôle of the taurocholate in this connection has been discussed. It is of especial interest to note that after the injection of sodium dehydrocholate when taurocholate has been replaced almost completely by dehydrocholate, the total ionic concentrations of serum and bile agreed closely. It would appear, therefore, that solutions of dehydrocholate show normal osmotic activity in contrast with those that contain taurocholate.

The effects of sodium cholate injection are shown in table 3. While the total cholate concentration of bile rose moderately, there occurred a significant decline in chloride and a marked decrease in bicarbonate. The reaction became more acid. This experiment was carried out before the importance of the osmotic pressure variations was apparent to us and no

figures for sodium concentrations are available. However, the considerable decrease in the sum of the concentrations of bicarbonate, chloride, and cholate may be contrasted with the increase in the total molar concentration of these ions after the injection of taurocholate. The action of unconjugated cholic acid in this respect resembled that of dehydrocholic acid. It seems probable that these unconjugated acids alike differ from taurocholic acid by exhibiting a normal osmotic behavior in bile.

SUMMARY

After intravenous injection of sodium taurocholate into a bile fistula dog, the total cholate and sodium concentrations increased in the bile while bicarbonate decreased. The total anion-cation concentration of the bile rose to a figure that exceeded considerably the total anion-cation concentration of serum. Such an effect is in accord with the view that taurocholate either exerts little osmotic pressure or lowers osmotic pressure by inactivation of inorganic ions.

Injection of sodium dehydrocholate caused the total anion-cation concentration of bile to diminish to values approximately the same as those observed in serum. The action of sodium cholate in this respect resembled that of dehydrocholate and differed from that of taurocholate.

The striking choleretic action of dehydrocholate is accomplished primarily by augmented excretion of water. A marked increase in the undetermined anion concentration of bile following the injection suggested that dehydrocholate was rapidly eliminated. The cholate (chiefly taurocholate) of dog bile was largely replaced by dehydrocholate. Total solids of bile decreased after injection of small amounts of sodium dehydrocholate, but remained constant or increased after the larger injections, due probably to the excretion of greater quantities of the injected material.

REFERENCES

Adler, A. 1929. In Handbuch der normalen und pathologischen Physiologie, vol. 4, 790, Berlin.

Chabrol, E. and M. Maximin. 1928. Compt. rend. Soc. biol., **99**, 1904.

Faludi, F. 1928. Zeitschr. ges. exp. Med., **61**, 121.

Greene, C. H. and A. M. Snell. 1928. Journ. Biol. Chem., **78**, 691.

Hammarsten, H. 1924. Biochem. Zeitschr., **147**, 481.

Horsters, H. 1932. Ergebn. d. Physiol., **34**, 494.

Jenke, M. 1931-2. Arch exp. Path. u. Pharm., **163**, 175.

Kauftheil, L. and E. Neubauer. 1932. Arch. exp. Path. u. Pharm., **166**, 675.

Regan, J. F. and O. H. Horral. 1932. This Journal, **101**, 268.

Stadelmann, E. 1891. Der Icterus und seiner verschiedenen Vormen. Nebst Beitragen zur Physiologie und Pathologie der Gallensekretion. Stuttgart.

Wakefield, E. G., H. P. Powelson and C. S. McVicar. 1929. Ann. Int. Med., **3**, 572.

THE EFFECT OF GLUCOSE DERIVATIVES UPON ANIMALS (RABBITS) FOLLOWING HEPATECTOMY

DOUGLAS R. DRURY AND WILLIAM T. SALTER

From the Medical Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University

Received for publication October 6, 1933

Mann and Magath (1) demonstrated that in dogs, from which the liver had been removed, there was a steady drop in blood sugar until the animal became moribund and died in convulsions. These investigators showed that the intravenous injection of glucose, mannose, maltose, and glycogen revived such animals when approaching death. Subsequently, Drury and McMaster (2) determined quantitatively the glucose requirement of hepatectomized rabbits and thus offered a basis for measuring the availability of carbohydrates other than those mentioned. The purpose of the work here reported was to determine how alterations in the molecular constitution of glucose would affect its utilization, as judged by its ability to prolong the life of a liverless rabbit.

An investigation of a similar sort was made by Herring, Irvine and Macleod (3), who dealt with the ability of glucose-derivatives to alleviate the symptoms characteristic of insulin hypoglycemia. Their results were complicated by the possible transformation of such compounds into glucose by the liver. In general, however, they demonstrated the marked specificity of glucose per se as an antidote for insulin. Meyer, McTiernan, and Salter (4), following their lead, tested the utilization by surviving tumor tissue not only of hexoses but also of so-called carbohydrate intermediates. The present paper reports analogous experiments with hepatectomized animals.

Experimental methods. The liver was removed from rabbits by a modification (5) of the Markowitz and Soskin (6) technic. This method requires the preliminary operation of partial occlusion of the portal vein and vena cava. As a result, large collaterals to these veins develop so that after several weeks the vessels may be completely ligated without embarrassing the circulation of the animal. With the portal vein and vena cava tied, hepatectomy is accomplished simply by cutting the attachments of the liver and removing it.

Liverless animals require the continuous administration of glucose postoperatively to prevent the development of hypoglycemia. It has been

found that rabbits fasted for two days before the hepatectomy use up glucose at a rather constant and uniform rate of approximately 125 mgm. per kilo per hour during the first ten to fifteen hours (2). This rate of injection must be maintained to keep the blood sugar level normal. If glucose be withheld, as in our control animals, the blood sugar drops 20 to 25 mgm. each hour, so that the animal becomes prostrated in about three hours, lapses into coma, and dies in about three and one-half hours. This

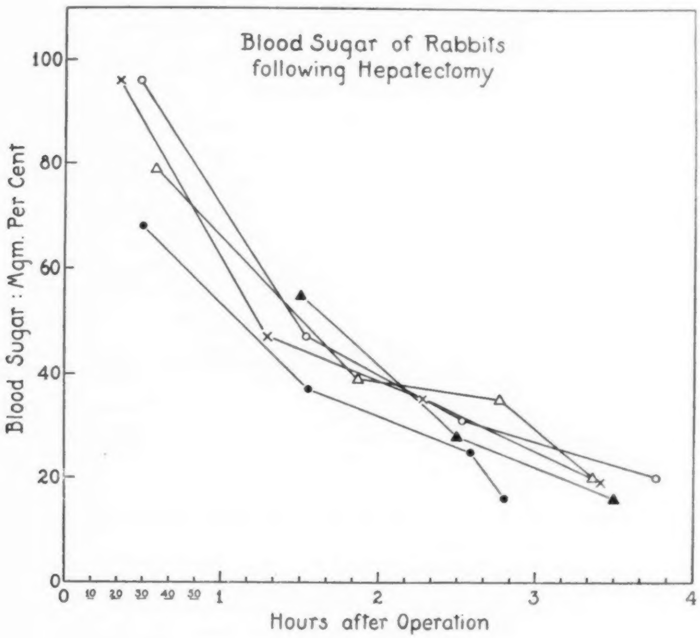


Fig. 1

is illustrated in figure 1. The "prostration time" ordinarily precedes the agonal convulsions by about 20 minutes.

In order to ascertain the probable concentration of glucose in the blood of control animals near exitus, the fermentable reducing substance for both plasma and whole blood was determined in two rabbits. The first of these was made three minutes before convulsions appeared, the second at cessation of respiration. The results were obtained through the kindness of Dr. A. Svedberg and appear in table 1. These values were found in unlaked blood (7) determined by the Folin and Svedberg fermentation method (8). The probable blood glucose, then, when convulsions occur

is about 15 mgm. per cent. Although the values given in figure 1 for total reducing substance are a little higher than the probable glucose, this discrepancy is important only near the end of the curves.

Such animals may be used to determine the action in the body of carbohydrates other than glucose. For this purpose we injected intravenously at regular intervals the particular carbohydrate to be tested and then determined the effect on the prostration time, on the survival time of the animals, and on the blood sugar. The amounts used were molecular equivalents of the expected glucose requirement, except that two molecules of triose were used for each glucose molecule. With some sugars the question of toxicity had to be considered. It was necessary to decide whether the poor condition of the animals after the carbohydrate had been injected for two hours or so was due to hypoglycemia or to a harmful action of the particular sugar used. In this case, a definite amount of glucose was injected intravenously and if the animal quickly resumed the

TABLE 1

Blood sugar of liverless animals

	WHOLE BLOOD			PLASMA			REMARKS
	Before fermentation	After fermentation	Apparent glucose	Before fermentation	After fermentation	Apparent glucose	
Rabbit 1	19	9	10	23	10	13	Before convulsions
Rabbit 2	22	6	16	30	9	21	After convulsions, at exitus

normal posture and behavior it was concluded that the previous condition had been due to hypoglycemia.

Correlation of experimental data. In such experiments, it was obviously necessary to correct the survival time for the glucose given. It is easy to estimate, from the dose of glucose given and from the animal's weight, the period of time that the life of the animal would be extended by a given injection: assuming that such animals uniformly require 125 mgm. of glucose per kilo per hour. If this period of time be subtracted from the actual survival time of the animals, the "corrected" survival time is obtained, which may be compared directly with that of the control animals. Such a procedure is valid because of the remarkably constant glucose utilization rate of such animals. This method was followed in the case of some of the sugars studied, as shown in table 2. The estimated values are enclosed in parentheses.

The glucose in these instances was not administered until the animal had become prostrated. Inasmuch as prostration ordinarily precedes death by about twenty minutes, it is possible also to estimate the survival

TABLE 2

*Survival time of liverless rabbits**

SUGAR	SURVIVAL TIME		REFERENCE TO UTILIZATION (SEE BIBLIOGRAPHY)
		hours	
Hexose-derivatives:			
Glucose		20.0	
Sorbitol		4.3	11, 12
Ethyl glucoside		(3.8)	13
		6.7	
Gluconic acid		4.2	13, 14
		(5.4)†	
Glycuronic acid		(3–)	15 (negative)
		(3–)	
Gulonic acid		3.3	16 (negative)
Saccharic acid		(2.6)	17
Glucosamine		(3.3)	17, 18
Fructose		(11.9)	19
Glucosone		2.5	20, 21
3-methyl glucose		(2.3)	
Thioglucose		4.8	
Gamma glucoses:			
Glucose (1, 4) monocarbonate		(2)‡	
		0.7‡	
Pringsheim's (1, 6)		2.0	
Triose derivatives:			
Glycerol		(3.8)	22, 23, 24
Glycericaldehyde		(3.5)§	24, 25, 26
Pyruvic acid		(2.7)	27, 28
	6.7	(6.7)	
Propylene glycol (1, 2)	3.3		13
	3.4	(5.3)†	
Dihydroxyacetone		2.7	
"oxantin"		(8.5)	25, 29, 30, 31
dimolecular		3.3	
Ethyl alcohol		3.2	
Ethylene glycol		(2.6)	32, 33
Controls 5		2.6	
315b		3.5	
15		2.8	
3		3.8	
32		2.6	
73		4.7	

* Corrected results in parentheses denote values adjusted for glucose administered as described in the text.

† Eviscerated.

‡ Toxic.

§ Corrected from prostration time.

time on that basis. In this manner the estimated survival time can be checked from the prostration time. When this test was applied to our calculations, it was found that in no case did the estimated time fall short of the observed time of prostration, but usually approximated it closely. This fact indicates that our corrected survival times (given in parentheses) are accurate to within twenty minutes.

Experimental results. In table 2 are presented, also, the respective survival times for control animals. Blood sugar analyses were not all

TABLE 3

Blood sugar of liverless animals (rabbits)

	TIME AFTER REMOVAL OF LIVER					
	0.5 hr.	1 hr.	2 hr.	2.5 hr.	3.0 hr.	3.5 hr.
Control 3	96	70	37	31		22
15	78	58	32	26		
315b		(70)	42			16
5				16		
32		72	26	20		
Hexose-derivatives:						
Sorbitol		66	46	39		33
Ethyl glucoside	81			40		28
Gluconic acid	81		48		37	33
	93		44	36		27
Gulonic acid	91		43	33	25	
Saccharic acid	84		40			
Triose-derivatives:						
Glycerol		74		44		24
Pyruvic acid		50	26			
Propylene glycol			60		43	37
			75	63	59	
	81		41	32	24	
	88	63	37		23	
Ethyl alcohol	71		31	26		

made at the same uniform intervals after operation. Interpolation was necessary, therefore, in order to compare the various animals in our series at similar stages. The fall of apparent blood sugar concentration, interpolated from the experimental determinations for hourly and half-hourly intervals, is shown in table 3. The substances used are presented in two groups, i.e., 1, the hexose derivatives, and 2, the trioses.

The net result of the hexose-derivative experiments is their failure to prolong the life of hepatectomized animals. In some instances there seems to be slight prolongation over the average control time, but the difference

is scarcely significant. A notable exception was fructose, but as Mann (1) has already pointed out, this effect disappears when the animal is eviscerated. The efficacy of this sugar, therefore, is probably due to its conversion to glucose through the agency of some abdominal organ or bacteria therein.

Despite the administration of these hexose derivatives, the blood sugar values closely approximate those of control animals provided the substance administered does not reduce the reagent. In general, the same was true for trioses. In the case of propylene glycol and of dihydroxy-acetone, anomalous results were encountered in an unusual prolongation of life, but this result could not be consistently obtained. The effect persisted after removal of the gastro-intestinal tract. A definitely prolonged survival time was twice obtained with the commercial dihydroxyacetone known as "oxantin." Despite its seemingly beneficial effect, each injection was accompanied by abnormal activity on the part of the rabbit suggesting a toxic effect. With the pure di-molecular dihydroxyacetone prepared according to Reeves and Renborn (9), neither toxic effect nor prolongation of life was evident.

DISCUSSION. *Mode of death.* Unless glucose be given, the hepatectomized rabbit dies when his blood sugar approaches 20 mgm. per cent or lower. Exitus is heralded by coma and by convulsions; followed by cessation of respiration, although the heart continues to beat. Even after the first convulsion, the intravenous injection of glucose may restore the animal to temporary well-being. It would appear, then, that glucose is necessary for some essential tissue, possibly the respiratory center. This center may require glucose for its metabolism, just as most centers require oxygen; that is, it may be constantly utilizing this compound, and when glucose is not available, death results. On the other hand, glucose might be necessary for the environment of the center just as calcium is necessary for the environment of this or some other center. The inability of the tested substances either to maintain blood glucose or to prolong life excludes both possible modes of usefulness for these compounds.

A striking feature of the inefficacy of the carbohydrates studied is the fact that most of them can be used by the intact animal. In table 2 are presented references to the bibliography of extant literature dealing with utilization of each respective sugar derivative. The evidence is based in some cases upon the formation of glycogen or upon the prevention of insulin-hypoglycemia; in other cases upon increased elimination of glucose in animals suffering from diabetes (mellitus or phlorizin). Hepatectomy, in short, removes the ability of the animal to convert various sugar derivatives into glucose. These results indicate that the liver is readily capable of chemical syntheses of a complicated sort, and suggest that a change in such function might be utilized as quantitative evidence of differential or specific liver damage.

Gamma glucose. It has been suggested by several investigators (10) that d-glucose, 1:5, is not the physiological form of dextrose, but rather that the "gamma" glucoses are used by living cells. The failure of the gamma forms to revive hepatectomized animals, however, (both the furanose, 1:4, form and the 1:6 form of Pringsheim) is distinctly against this view. It would seem that the mammalian tissues observe a specificity for normal dextrose, i.e., gluco-pyranose, 1:5.

Carbohydrate intermediates. In addition to lactic acid, several triose derivatives, e.g., glyceric aldehyde, dihydroxy-acetone, glycerol, and pyruvic acid, have been suggested by various authors as intermediary substances in the metabolism of glucose. That they undoubtedly can be precursors of glucose is established by the increase in liver glycogen following the feeding of them to fasted animals (25, 26, 28), by the relief or prevention of insulin convulsions when injected into the experimental animal (12, 23, 29), and by the increase in glycosuria following their administration to phlorizinized and diabetic animals (19, 24, 31).

The intact animal is able not only to convert these substances into glucose but also, in some cases, to change glucose into them. This is well known in the case of lactic acid, itself a triose derivative. That the liverless animal is unable to carry out the former of these processes is indicated by the fact that when these substances are injected into liverless animals the blood sugar does not rise and hypoglycemic death supervenes in the usual time. The rôle of trioses as carbohydrate intermediaries would seem to be a distinctly limited one in the light of these experiments. As far as extra-cellular carbohydrate is concerned, they would seem to be of no more importance to the intact animal than are those hexose derivatives which the liver converts to glycogen.

SUMMARY

A number of hexose-derivatives and triose-derivatives have been tested for their ability to substitute for glucose in prolonging the lives of rabbits following removal of the liver. Although the intact mammal can transform a number of such carbohydrates to glycogen or glucose, the hepatectomized animal is not benefited by many simple derivatives of glucose, even those involving substitution of only a single chemical group. The transformation of these substances into glucose by the intact animal is evidence of a complex, synthetic chemical function of the liver.

Although the so-called carbohydrate intermediates can be substituted for glucose in the intact animal, they serve no obvious purpose in the hepatectomized animal. This fact raises serious doubts as to their being an important form of exchange of carbohydrate between various tissues or organs.

REFERENCES

(1) Mann, F. C. and T. B. Magath. Arch. Int. Med., 1922, **30**, 171.
(2) Drury, D. R. and P. D. McMaster. Proc. Soc. Exp. Biol. and Med., 1929, **27**, 48.
(3) Herring, P. T., J. C. Irvine and J. J. R. Macleod. Biochem. Journ., 1924, **18**, 1023.
(4) Meyer, O. O., C. McTiernan and W. T. Salter. To be published.
(5) Drury, D. R. Journ. Exp. Med., 1929, **49**, 759.
(6) Markowitz, J. and S. Soskin. Proc. Soc. Exp. Biol. and Med., 1927, **25**, 7.
(7) Folin, O. Journ. Biol. Chem., 1930, **86**, 176.
(8) Folin, O. and A. Svedberg. Journ. Biol. Chem., 1926, **70**, 405.
(9) Reeves, H. S. and E. T. Renborn. Biochem. Journ., 1931, **25**, 411.
(10) Irvine, J. C. Ind. and Eng. Chem., 1923, **15**, 1162; Chem. Rev., 1927, **4**, 203.
(11) Embden, G. und W. Griesbach. Zeitschr. f. Physiol. Chem., 1928, **91**, 251.
(12) Grafe, E. und F. Meythaler. Arch. f. Exper. Path. u. Pharm., 1928, **136**, 360.
(13) Salter, W. T. und P. D. Robb. To be published.
(14) Paderi, C. Arch. ital. biol., 1915, **64**, 81.
(15) Biberfield, J. Biochem. Zeitschr., 1914, **65**, 479.
(16) Paderi, C. Chem. Zentralbl., 1911, **1**, 1370.
(17) Paderi, C. Arch. form. sper., 1916, **22**, 96; Chem. Abst., 1916, **10**, 2760.
(18) Moschini, A. Arch. ital. biol., 1924, **74**, 117.
(19) Deuel, H. J. and W. H. Chambers. Journ. Biol. Chem., 1925, **65**, 7.
(20) Hynd, A. Proc. Roy. Soc., London, B, 1927, **101**, 244.
(21) Dixon, K. C. and K. Harrison. Biochem. Journ., 1932, **26**, 1954.
(22) Chambers, W. H. and H. J. Deuel, Jr. Journ. Biol. Chem., 1925, **65**, 21.
(23) Voegtlin, C., E. R. Dunn and J. W. Thompson. Journ. Pharm., 1925, Proc. **25**, 168.
(24) Fischler, F. Zeitschr. Physiol. Chem., 1927, **165**, 68.
(25) Embden, G., E. Schmitz and M. Wittenberg. Zeitschr. Physiol. Chem., 1913, **88**, 210.
(26) Stohr, R. Zeitschr. Physiol. Chem., 1932, **206**, 211.
(27) Dakin, H. D. and N. W. Janney. Journ. Biol. Chem., 1913, **15**, 177.
(28) Otani, Z. Zeitschr. Physiol. Chem., 1925, **143**, 229.
(29) Reeves, H. G. and J. A. Hewett. Proc. Physiol. Soc., Journ. Physiol., 1926, **25**, 61.
(30) Campbell, W. R. and J. Hepburn. Journ. Biol. Chem., 1926, **68**, 575.
(31) Ringer, A. I., and E. M. Frankel. Journ. Biol. Chem., 1914, **18**, 233.
(32) Barrenscheen, H. K. Biochem. Zeitschr., 1913–14, **58**, 277.
(33) Franck, H. Münch. Med. Wochenschr., 1918, **65**, 1216.

CHANGES IN VOLUME AND VELOCITY OF BLOOD FLOW IN CHRONIC EXPERIMENTAL AORTIC REGURGITATION AND THE EFFECT OF CERTAIN DRUGS IN THIS CONDITION

W. F. HAMILTON, IRVING BROTMAN AND GEORGE BREWER

From the Departments of Physiology and Medicine, George Washington University Medical School

Received for publication August 2, 1933

Work with the Brilliant Vital Red dye injection method (1) in measuring volume and velocity of blood flow in normal animals under the influence of certain circulatory drugs (2) has led us to perform parallel experiments on animals with surgically produced chronic aortic regurgitation.

A series of 15 determinations on the normal dog is reported here and elsewhere (2). These experiments show that on the average the lesser circulation time is 7.7 seconds, the mean circulation time is 14.7 seconds and the total circulation time is 13.4 seconds. All of our experiments were done after a tranquilizing dose of morphine which reduced the average pulse rate to 63. The cardiac output or blood flow (F) averaged 5.1 liters per square meter (6) per minute. The volume of actively circulating blood in the heart, lungs and great vessels (V), averaged 1.2 liter per square meter. The stroke volume averaged 84 cc. per square meter and the ratio V/SV proved to be 15, i.e., fifteen heart beats were required to move 1.2 liter (V) of blood through the circulation. It will be noticed that all figures relating to volume are given in terms of surface area (6), and are to be understood as being per square meter of body surface whether this is explicit or not. In this paper we shall study the factors just mentioned in relation to the valvular lesion and in relation to stresses put upon the cardiovascular system by massive doses of certain drugs. It is to be distinctly understood that we approach this problem of drug action not as pharmacologists in evaluating therapeutic dosage, but rather as physiologists undertaking to stimulate, strain or disarrange certain organs and processes and to observe the results and their compensating mechanisms.

Elsewhere we are presenting the changes which occur in the transmission pulses as studied by a new hydrogen pulse recorder (3) and the pressure pulses (4) as studied by a new hypodermic manometer (5).

The operation which was performed in identical fashion on all of the animals resulted in a series of lesions of varying severity. The valves were

ruptured by thrusting a stylet down the carotid artery and through the aortic cusps.

At the time of the operation dog D showed a sudden increase in heart rate, a faint diastolic murmur, pistol shot pulse and an increase in pulse pressure as estimated by the Korotkow sounds. A few days later, however, the animal showed none of these clinical signs.

The lesser circulation time was found on two occasions to be 8.3 and 7.9 seconds as compared with 8.5 seconds before the operation. The mean circulation time was within normal limits, 13.3–14.4 seconds as was the total circulation time. The pulse, 52–58 beats per minute, was of the same order as would be expected in a normal dog under the experimental conditions.

All volume figures (6) show slight changes of doubtful significance. *F* which was 6.7 liters per square meter per minute has been reduced to 5.5 and 4.0, figures which are not out of the normal range but are low for this dog. *V* has been reduced from 1.6 to 1.2 and 0.95L. The stroke volume before the operation was 103 cc. and in two subsequent experiments 106 and 68 cc.—an insignificant change. The ratio V/SV remained within the normal range 11–16.

X-ray and electrocardiographic examination revealed no clear cut change. The pathologic report (autopsy 5 months later) showed an essentially normal heart with a scar where a lesion in one of the aortic cusps had healed. A post-mortem functional test indicated that the valve did not leak.

The effect of epinephrine (0.5 mgm.) in this animal was to reduce the stroke volume from 103 to 30 cc. This is a parallel finding to that obtained in the unoperated dog where the effect of epinephrine is to reduce this figure to an average of 35 cc. The heart rate on the other hand instead of being reduced to 60 or thereabouts by the depressor mechanism was increased (direct drug effect on the heart) to 140. An interference with the depressor mechanism is here noted which is even more striking in the other members of this series. The fast heart rate brought the minute volume to a larger figure (4.1) than for the normal epinephrine series (2.2) and at the same time hastened the velocity flow. (M.C.T. = 20.3 seconds as compared to 37.6 in the normal.)

Sodium nitrite (195 mgm.) which is less effective than amyl nitrite produced no change in this dog.

Acetyl-beta-methylcholine-chloride (2 mgm. intramuscularly) quickened the velocity flow (M.C.T. = 5 seconds) and probably increased the volume flow. The effect of this drug will be reported upon separately (7).

As shown by the early clinical findings and by the scar at autopsy, the aortic valve was damaged at operation. That a certain amount of healing occurred was shown during life by the disappearance of the early clinical

signs after a few weeks and by the fact that the injection method results quickly became essentially normal. The existence of some permanent damage was indicated by the fact that the response to cardiovascular strain (epinephrine) was abnormal.

The next animal in this series, dog E, responded to the operation as did dog D, except that the signs were more pronounced and persisted for two months, that is, until death occurred accidentally. X-ray examinations showed no change from the normal. Electrocardiographic evidence showed obscure changes in rhythm and conduction but no clear evidence of myocardial damage.

By the injection method it was found that the stroke volume showed no change. *F* changed from 4.2 to 3.3, two weeks later increasing to 5.7. *V* decreased from 1.2 to 0.9, returning subsequently to 1.2.

Epinephrine in this animal caused a response wholly different from that in the normal series. Stroke volume was reduced from 60 to 7 cc., one-fifth the usual epinephrine figure (2). Vagus influences seemed to be entirely absent and heart rate increased to the surprising figure of 220 beats per minute. In spite of this rapidly beating heart *F* is reduced to 1.5 liters per minute. The mean circulation time (61.4 seconds) is double that in the normal epinephrine series which in turn is double that in the group without the drug. *V*, 1.5 liter, is increased 50 per cent and the ratio of V/SV, that is, the number of heart beats required to move this bulk of blood out of the chest, is increased from 15 to 224.

Amyl nitrite produced little or no effect on these factors.

The responses of this animal were to a type of lesion intermediate in severity between dog D and those yet to be described. The responses soon after the operation were more abnormal than those of dog D and during stress (epinephrine) were of the same order as those of the animals with more extensive lesions.

Dogs A and F differ from E in that the lesions were much more severe, giving rise to intense and typical signs of Corrigan's disease which continued until death. However these dogs never showed any clinical evidence of decompensation,—edema, dyspnea at rest or râles at the lung bases.

Electrocardiographic evidence in dog A showed marked immediate myocardial disturbance followed by evidence of left ventricular preponderance. By x-ray this animal showed enlargement of the heart. At autopsy five months after operation the dog showed a large aortic leak (524 cc./minute at 62 cm. water pressure). The cavity of the left ventricle was greatly enlarged and that of the right nearly obliterated (10). The walls of the left ventricle were markedly hypertrophied, those of the right thin and atrophic. The left auricle was increased in size and its walls thickened, while the right auricle was atrophied. The A-V valves

were normal but one cusp of the aortic valve was almost completely detached.

The electrocardiogram on dog F showed the same picture as dog A. Repeated x-ray examination however failed to show any enlargement.

At autopsy the aortic leak measured as before amounted to 660 cc./minute.

Pathologic examination of the heart of this animal showed moderate right auricular dilatation, relative tricuspid insufficiency with fibrosis of the margins of the valve and fibrous adhesions to the septum. The left auricle was dilated and hypertrophied and one cusp of the aortic valve had a large fenestration. There was hypertrophy of the wall of the left ventricle but this was less marked than in the case of dog A. There was no dilatation.

By the injection method dog A showed as a result of the operation a reduction in stroke volume from 69 to 28 cc. After a period of four months this figure had returned to 42 cc. Dog F followed a similar course.

The minute volume made parallel changes in both dogs being reduced by operation from 4.7 to 2.5 liters in dog A and from 4.2 to 2.7 liters in dog F. In both cases there was a subsequent return nearly to normal. The velocity flow made no significant change. The pulse is accelerated from 68 to an average of 90 in dog A and in dog F from 52 to an average of 92.

The ratio of V/SV in dog A changed from 22 to 31 and in dog F from 14 to 25.

V, surprisingly enough, showed a lower value after the operation (average 0.85) than before (average 1.3), returning to a more usual figure in both dogs later. We had looked for this factor to increase instead of decrease, since the handicapped left ventricle would be expected to dilate and exert back pressure on the lungs so as to produce congestion. It is not impossible that the encroachment upon the cavity of the right ventricle by the dilating left explains the adjustment of right heart output to left heart output without pulmonary congestion (8). This notion must be applied with considerable caution since the right ventricle of dog F was not encroached upon. The rôle of the unexpected tricuspid lesion adds to the puzzle. The function of the pericardium in this adjustment would be an interesting one to explore.

The effect of epinephrine in these animals with severely damaged hearts was similar to the effect already described in dog E, the mean circulation time being increased to 94 and 67 seconds in dogs A and F respectively and the dye concentration curve prolonged and flattened as in human cases of severe cardiac decompensation (1 and 2). The heart rate is increased in the case of dog A to 270 beats per minute and the stroke volume reduced in the same experiment to 3.5 cc. In dog F similar but less extensive changes occurred in pulse rate and stroke volume. In general there is an increase in pulmonary congestion as evidenced by the increase in V; in the

case of dog A from 0.87 to 1.5 and in the case of dog F from 0.8 to 2.1. This increase coupled with the low value of F (0.9 in dog A and 1.9 in dog F) makes it seem possible that there is blood completely stagnating in the pulmonary tree and hence not included in V (1, 2, 9). The tremendous increase in the ratio of V/SV indicates that as many as 420 heart beats are needed to move 1500 cc. of blood out of the chest.

Vasodilatation as produced by nitrites hastened the circulation, increased the volume flow and reduced the volume of blood in the heart, lungs and great vessels so that these quantities became more nearly normal. In this same regard acetyl-beta-methylcholine chloride was found to be a much more potent drug, even in doses as small as 1 mgm., intramuscularly, in that these quantities were moved from their values in aortic regurgitation not only to the normal figures but beyond these values.

It is our pleasure to acknowledge the help of Dr. Chester E. Leese of this department in some of the experiments and Dr. R. M. Choisser of the department of Pathology for the pathological description of the hearts. We are grateful to Dr. Isaac Starr, Jr., of the University of Pennsylvania for our supply of acetyl-beta-methylcholine chloride.

SUMMARY

An analysis by means of the injection method of chronic experimental aortic regurgitation showed changes in the relationships between cardiac output (F), stroke volume (SV), circulation times (lesser, greater and mean) and the volume of actively circulating blood in the heart, lungs and great vessels (V).

Animals with lesions of varying severity were prepared and their responses to the lesion itself, to decompensating doses of adrenalin, and to vasodilator drugs were studied over a period of months.

The clinical picture of Corrigan's disease was produced. The signs corresponded in intensity with the degree of change as determined by the dye injection studies and with the severity of the lesion as found at autopsy.

In the more severe cases there was electrocardiographic evidence of myocardial damage and left ventricular preponderance, and in one case evidence by x-ray of decided enlargement.

The effect of the lesion was to decrease the measurements of F, SV and V and to increase heart rate, and the ratio V/SV. These changes were in proportion to the severity of the lesion and tended to return toward normal with the passage of time. The method of this last change was either by simple healing, by hypertrophy of the left ventricle or by hypertrophy with marked dilatation.

In regurgitation, the effect of temporarily decompensating doses of epinephrine is to reduce F and SV to a greater degree than in the normal.

V on the other hand is increased, and the striking absence of the depressor reflex causes a large immediate increase in heart rate, not seen in the normal dog.

Vasodilator drugs, nitrites and acetyl-beta-methylcholine chloride cause increases of varying amounts in the volume and velocity flow. These drugs may carry the above measurements and the ratio V/SV to or beyond their value before operation.

REFERENCES

(1) Hamilton, W. F., J. W. Moore, J. M. Kinsman and R. G. Spurling. This Journal, 1932, **99**, 534.
(2) Hamilton, W. F. This Journal, 1932, **102**, 551.
(3) Brewer, G., I. Brotman and W. F. Hamilton. This Journal, in press.
(4) Brewer, G., W. F. Hamilton and I. Brotman. This Journal, in press.
(5) Hamilton, W. F., G. Brewer and I. Brotman. This Journal, in press.
(6) Cowgill, G. R. and D. L. Drabkin. This Journal, 1926, **81**, 36.
(7) Brotman, I., G. Brewer and W. F. Hamilton. In press.
(8) Henderson, Y. and A. L. Prince. Heart, 1914, **5**, 217.
(9) Hamilton, W. F., J. W. Moore and J. M. Kinsman. This Journal, 1927, **82**, 656.
(10) Bazett, H. C. and J. Sands. Journ. Clin. Invest., 1926, **3**, 65.

ANALYSIS OF THE PULSE CONTOUR IN RELATION TO SOUND PRODUCTION BY MEANS OF THE HYDROGEN TRANSMISSION PULSE-RECORDER

GEORGE BREWER, W. F. HAMILTON AND IRVING BROTMAN

From the Departments of Physiology and Medicine, George Washington University Medical School

Received for publication August 2, 1933

In taking optical records of the femoral pulse of normal dogs it was found that conventional apparatus (1) was adequate. In the course of our work on aortic regurgitation, when the valves were torn (2) the femoral pulse became water-hammer in type and auscultation revealed a loud "pistol-shot." Records of this type of pulse were entirely inadequate. The conventional apparatus was thrown into instrumental vibrations which completely overshadowed the true contour of the pulse curve. Our reason for thinking that these vibrations were instrumental is that as the tube length was changed the vibrations changed correspondingly in period. Figures 1 and 2 illustrate this observation.

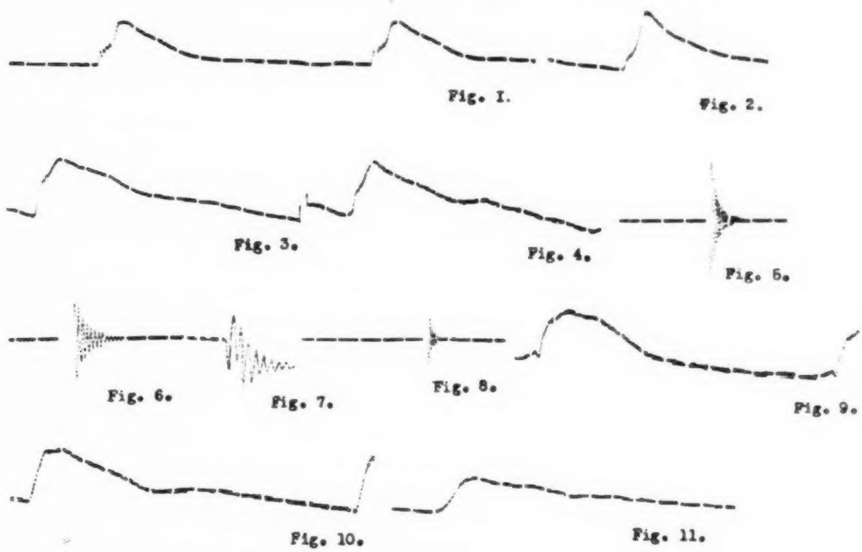
The spring thread recorder. In order to eliminate these artefacts we constructed a spring pulse recorder. A very light cantilever spring was placed over the artery so that its movements were imparted to the spring. The movements of the spring were in turn transmitted over a fine silk thread of adjustable tension to the recording mirror. This latter was mounted upon a similar spring and the apparatus as a whole had a frequency which depended upon the tension of the thread but was, under working conditions, above 500 cycles per second.

This apparatus gave a pulse curve which faithfully followed the movements of the artery. A record taken by the spring thread recorder is shown in figure 3. It shows a very sudden upstroke from the diastolic level. In some of these dogs with regurgitation there was a negative wave or notch immediately preceding this upstroke. The upstroke is sometimes followed by a short, step-like plateau occupying two or three sigmata, which in turn is followed by a smooth rounded upstroke that merges into the rounded summit of the curve. Usually the step-like plateau does not appear, and occasionally the initial sudden upstroke carries the curve directly to the summit. There is nothing noteworthy about the descent except the flattening of the dicrotic notch.

Particular note should be taken of the fact that there are no up and down

vibrations anywhere on the curve. In this sense it is smooth, and the movements are very quick.

The hydrogen recorder. While perfectly satisfied with the accuracy of records taken with such a spring recorder, its inconvenience and the time consumed in making the necessary adjustments justified the attempt to imitate these records with an apparatus using the principle of gas transmission. The chief improvement necessary is to increase the frequency of the conventional transmission recorder. Obviously the recording capsule



Figs. 1 to 11

Fig. 1. Tube length 100 cm. Vibration of artefacts 140/second. Dog A, aortic regurgitation time in 1/20 second in all figures.

Fig. 2. Tube length 64.5 cm. Vibration of artefacts 200/second. Dog A, aortic regurgitation.

Fig. 3. Spring thread pulse recorder. No artefacts ($N = 500^{+}$). Dog A, aortic regurgitation.

Fig. 4. Hydrogen pulse recorder. ($N = 900^{\pm}$). Dog A, aortic regurgitation.

Fig. 5. Tube length 64 cm. Receiving cup in firm contact with skin ($N = 200^{+}$).

Fig. 6. Tube length 64 cm. Receiving cup covered with tense rubber dam ($N = 100^{+}$).

Fig. 7. Tube length 250 cm. air filled ($N = 50^{\pm}$).

Fig. 8. Tube length 250 cm. H_2 filled ($N = 200^{\pm}$).

Fig. 9. Taken from brachial artery during compression of upper arm between systolic and diastolic pressures. Loud, sharp sound on auscultation. Hydrogen recorder ($N = 800^{+}$).

Fig. 10. Same as figure 9. Below diastolic pressure. Muffled thud on auscultation.

Fig. 11. Same as figures 9 and 10. Pressure 0. No sound on auscultation.

must have a sufficiently tense membrane and light construction to possess of itself an adequately high natural frequency. Having the membrane sufficiently tense to raise its frequency to 800 or 900 cycles per second may reduce the sensitivity so that it is insufficient at the ordinary photographic distance of 1 meter. Using the optical system described elsewhere (3), sensitivity may be increased by increasing the photographic distance up to 5 m.

When the recording capsule has a sufficiently low period we have found by experiment that the frequency inscribed by the apparatus as a whole is a function only of the tube length and can be calculated by the formula $N = \frac{V}{2L}$ where N equals the frequency in cycles per second, V the velocity of sound in centimeters per second (about 28,000 according to our measurements for tubes of this size, and also according to those of Bramwell, Hill and McSwiney (4)) and L is the length of the air column in centimeters.

This analysis applies only when the tube is functioning after the manner of a closed pipe, i.e., when the receiving cup is pressed firmly against the skin. On the other hand, when one transmits the pulse by means of a button to a tense membrane over the receiving cup (see (1), p. 194) this formulation does not hold. The natural frequency of the system is halved, and the air column resonates as it would in an open pipe, i.e., $N = \frac{V}{4L}$. Figures 5 and 6 illustrate this point quite conclusively. Due to its sluggishness of movement the glycerine pelotte was found decidedly less satisfactory than the simple cup in close contact with the skin.

This argument leads to the conclusion that by shortening the tubing one can reduce the period to such a value that no after vibrations should appear on the record. As a matter of fact, however, when the tube length is reduced to about 30 cm. after vibrations whose period corresponds to the tube length occasionally vitiate the records. Now it is very inconvenient to work with tubes shorter than 30 cm. so it became necessary to fill the tube with a gas that transmits waves at a greater velocity than air. The best gas for this purpose is obviously hydrogen, in which the velocity of transmission is about four times that in air (from our observations in 5 mm. tubing about 110,000 cm. per sec.). In accordance with expectations the frequency inscribed by a recording system is increased fourfold by filling it with hydrogen. This is clearly illustrated by figures 7 and 8.

The above findings are directly contradictory to the predictions of Otto Frank who states (5) that no useful increase in the frequency of an air transmission sphygmograph accrues if the tube length is reduced to less than 64.5 cm. and further that replacing the air in such an apparatus with hydrogen will increase its frequency only 14 per cent.

Frank derives this prediction by applying, with few modifications, the

equations developed in analyzing manometer systems (6, 7). The concepts employed are the "effective mass" and "volume elasticity coefficient" of the several parts of the system. Since decreasing the length of the gas column or changing the gas from air to hydrogen works a relatively small decrease in the "effective mass" of the system Frank predicts only a small increase in the frequency when these changes are made.

As we see it, however, the recorded frequency is that of the slowest part of the system, usually the transmitting column of gas. By using hydrogen or by shortening the column of gas the frequency of the whole system can be increased up to the limit set by the natural frequency of the recording capsule. Capsules can easily be constructed with a frequency of 800 or 900 cycles per second and it is found by experiment that these frequencies can be attained by using hydrogen in tubes about 65 cm. long or air in tubes about one-fourth that long. These latter are of course inconvenient to use.

Like the records made by the spring thread recorder, those made by a rapid hydrogen recorder satisfy all the criteria which we can use in judging them. As seen in figure 4 there are no instrumental vibrations. The initial upstroke is quick, the amplitude adequate and the contour of the pulse conforms to that shown in figure 3.

Sound production by physiological pulsations. On auscultation of the pulse whose contour is given in figures 3 and 4, one hears a loud "pistol shot" sound. In view of the literature (8, 9, 10 and 11) it seems rather paradoxical to find sounds produced by "smooth" pulsations. One expects to see vigorous up and down vibrations and to think of these vibrations as explaining the sound. The record is however perfectly smooth and remains so even when the sensitivity of the apparatus is so increased that the pulsations are about 15 cm. high. If this sensitivity is used and a side tube is open there result vigorous "phonoarteriograms" of the usual vibratory type (8).

It is easy to imagine conditions under which the center of the tympanic membrane would execute movements closely analogous to those recorded in the pulse curve. These conditions would simply involve those of ordinary auscultation with an air tight connection between the pulsating area and the ear. Now if a side tube in the stethoscope is opened, the intensity of the sound is reduced but its quality remains unchanged, in spite of the fact that from the characteristics of the "phonoarteriogram" (made under analogous conditions) one might expect the sounds under the two conditions to differ as widely as the phonogram differs in appearance from the pulse tracing.

It seems to us that the reason for the similarity of the arterial sound under conditions where the pressure curves differ so widely is to be understood in terms of the fact that the ear is an harmonic analyzer of pressure

pulsations. The "phonoarteriogram," strictly speaking, partakes of the nature of a tachogram of the pulse and an harmonic analysis of the tachogram would show very similar higher (audible) components to an harmonic analysis of the pulse curve itself. This explains the similarity of the sounds heard through the stethoscope with side tube open and with the side tube closed.

It would seem further that one can gain little insight into the frequency and nature of the sounds produced by physiological pulsations from the usual arterio and cardio phonograms (1, 4, 8, 9, 10 and 11). Whether the pulsations are recorded electrically or directly, it would seem that fundamentally the "phonogram" is at least a partial tachogram of the pulsation. The tachogram of a curve is unfortunately not its harmonic analysis and the mixtures of tachographic curves, instrumental vibrations and resonance frequencies, which have been published as sound records are certainly harder to interpret as to the frequency and intensity of the harmonic components than would be the simple apex or pulse curve taken without the tachographic "improvement" of an open side tube.

Certain pulsations produce sound and some do not. In general our experience leads us to conclude that those with very quick strokes (either up or down) are those which produce sound. These pulsations will be found to show components of relatively high frequency and amplitude upon harmonic analysis. If the harmonic components reach the audible frequencies at high enough intensity the pressure wave will be audible.

These quick movements are such as throw the older recording systems into instrumental vibrations and give rise to such curves as those published by Feil and Gilder (10) and Bramwell (9). These vibrations are generally interpreted as real and representative of the sounds heard on auscultation. Vibrations of this character are seen in figure 1. That they are artefacts is shown by their dropping out when more efficient apparatus is used, see figures 2, 3, 4.

The contrast between pulsations which produce sound and those which do not is shown in figures 9, 10 and 11. These curves were obtained from the brachial artery during a series of experiments attempting to correlate pulse contour with the phases of the Korotkow sounds during decompression of the upper arm with the conventional blood pressure cuff. Figure 9 is a pulse curve with the pressure in the cuff half way between systolic and diastolic pressure. On auscultation of the cubital region with the unaided ear one hears a relatively high pitched sound under these conditions. The curve is taken with a hydrogen recorder whose frequency is above 800 per second and shows no instrumental vibrations, nor does it show any up and down vibrations of arterial origin. The sound is evidently made by the sudden movement of the artery and not by vibrations produced by anacrotic turbulence of the blood stream under the receiver

(9). Contrasting figure 9 with figures 10 and 11 it is seen on inspection that they show decreasing quickness of movement, and one may safely predict that the higher harmonic components of the three curves possess serially decreasing frequencies and amplitudes. The suggestion which we wish to record here is that in figure 9 these harmonic components are well into the audible range, in figure 10 they are near the threshold and in figure 11 they are below the threshold of audibility.

The apex-beat presents the same type of sound records in the case of the normal heart. There are two simple notches, corresponding to the valvular components of the two heart sounds. No repetitive waves appear on the record when taken with an instrument which is not thrown into resonance vibrations.

In cases of valvular abnormalities, however, it is possible to demonstrate a continued series of vibrations over either the heart or the arteries. These vibrations when of high enough frequency are audible as murmurs.

SUMMARY

1. Optical pulse recording systems of the conventional type are thrown into instrumental vibrations by quickly moving pulsations such as those in experimental aortic regurgitation, and hence do not give true records.

2. A "spring thread" pulse recorder (N = 500–600) was constructed and shown to give adequate records. Its inconvenience however interfered with its usefulness.

3. The air transmission sphygmograph was modified by using hydrogen as the conducting gas. The natural frequency of the apparatus was thereby quadrupled, contradicting Otto Frank's mathematical prediction of a mere 14 per cent increase. The frequency of our apparatus can easily be raised to 800 cycles per second and it will give adequate records of quickly moving pulsations.

4. In aortic regurgitation it is shown that the abrupt arterial sounds are produced by quickly moving smooth pulsations, whose contours lack entirely the repetitive vibrations ordinarily identified with the sound as heard. Similarly, the pulsations causing the normal heart sounds (valvular components at least) and Korotkow sounds are not characterized by repetitive vibrations.

5. Phonoarteriograms and phonocardiograms as ordinarily transcribed, directly or electrically, give a less adequate representation of the sounds as heard over the artery or heart, than would an harmonic analysis of the simple pulse curve or apex beat.

6. Such sounds as murmurs, however, appear on an adequate record as a series of vibrations.

We are grateful to Mr. R. L. Wegel of the Bell Telephone Laboratories for his helpful advice.

REFERENCES

(1) Wiggers, C. J. 1923. Modern aspects of the circulation in health and disease. Lea & Febiger, Philadelphia.
(2) Hamilton, W. F., I. Brotman and G. Brewer. This Journal, in press.
(3) Hamilton, W. F., G. Brewer and I. Brotman. This Journal, in press.
(4) Bramwell, J. C., A. V. Hill and B. A. McSwiney. Heart, 1923, **10**, 233.
(5) Frank, O. Zeitschr. f. Biol., 1929, **89**, 277.
(6) Frank, O. Zeitschr. f. Biol., 1903, **44**, 445.
(7) Frank, O. 1913. Tigerstedt's Handbuch der Physiologischen Methodik. II, 2 hälfte, 1.
(8) Bramwell, J. C. and S. K. Hickson. Heart, 1926, **13**, 129.
(9) Bramwell, J. C. and S. K. Hickson. Heart, 1926, **13**, 109.
(10) Feil, H. S. and M. D. D. Gilder. Heart, 1921, **8**, 4.
(11) Asher, A. B. Arch. Int. Med., 1932, **50**, 913.

PRESSURE PULSE CONTOURS IN THE INTACT ANIMAL

I. Analytical Description of a New High-Frequency Hypodermic Manometer with Illustrative Curves of Simultaneous Arterial and Intracardiac Pressures

W. F. HAMILTON, GEORGE BREWER AND IRVING BROTMAN

From the Departments of Physiology and Medicine, George Washington University Medical School

Received for publication August 2, 1933

In order to amplify the work with the injection method in relation to volume flow (1) and with the hydrogen pulse recorder in relation to the contour of the external pulses (2) we have decided to study the pressure pulse under the same conditions as those involved in the other two studies.

So as to avoid the inaccuracies of the auscultatory and oscillatory methods of determining blood pressure, and to eliminate the operations required for using the manometers of Frank (3) and Wiggers (4), it was found necessary to design a manometer in which the ideals of adequate sensitivity and very quick response (high frequency) were combined with the possibility of inserting the cannula (Luer needle) through the skin and into that part of the vascular system whose pressure pulses we desired to record.

Random experimentation resulted in a manometer whose body dimensions were of the same order as those heretofore described (3, 4). The cannula, however, was much smaller (5) (18 gauge Luer needle of length appropriate to the region to be explored). In place of the conventional rubber dam membrane we substituted a membrane of "shim" brass 0.06 mm. in thickness. This manometer was found to have a very high natural frequency and adequate sensitivity. In none of our work has the problem of obtaining sufficient sensitivity been a bothersome factor, as with our reflecting system (see below) the optical lever may be extended to 5 or 6 meters when such magnification is required (5, 6).

The need for improving the convenience of our apparatus led us to serious consideration of the equations of Frank (3, 7, see also 4, 8) describing manometer systems. We had discovered in a general way that the frequency of such apparatus seemed to vary with the size of the component parts, particularly with the size of the cannula. If the equations of Frank applied to manometers such as ours there would be at hand a most convenient method for predicting, for example, what decrease in frequency

could be expected on replacing an 18 gauge needle with a 20 gauge, or in extending the length of the manometer tube from 10 cm. to 25 cm.

According to Frank's formulation the frequency of a manometer system varies directly with its "volume elasticity coefficient" and inversely as its "effective mass." The effective mass of the manometer as a whole is the sum of that of its parts. Their combined mass, in turn, is determined from their length and diameter by the formula $M = \Sigma \frac{1}{\pi r^2}.S$ where M equals the effective mass, l equals length, R equals radius and S equals specific gravity of the fluid contained in the manometer. One should therefore expect to find that the smaller the diameter of the needle employed the greater would be the effective mass and accordingly the less the frequency.

The volume elasticity coefficient (E^1) is a factor depending only on the membrane employed and is described by the relationship $E^1 = \frac{\Delta P}{\Delta V}$ i.e., the change in volume under the membrane due to unit change in pressure impressed on the manometer system.

To test the validity of applying these concepts to manometers differing so radically from those for which they were developed (see table 1, last two lines) we constructed a series of manometers, 1-A, 1-B, 1-C, 1-D, having the same membrane and optical systems but widely differing effective masses. As seen from the agreement of the figures in the next to last column in table 1, calculated from Frank's formula, $\frac{1}{N} = 2\pi\sqrt{\frac{M}{E^1}}$, with those in the last column of actually determined frequencies, one is justified in accepting Frank's formulae as a basis for the design of manometers of the material and dimensions which we are using.

The chief difficulty in making estimations of frequency with manometers like ours is that small air bubbles may be trapped within the manometers, thereby adding to the volume elasticity coefficient of the system—so vitiating the frequency determination (figs. 2 and 3). This situation necessitates many frequency determinations at short intervals and would involve endless labor if the classical method (8, 9) were followed.

We fill the manometer with boiled citrate (3 per cent) which has been stored under oil, making every effort to coax possible bubbles out through the needle. The cannula is thrust through a thick rigid rubber stopper which closes one end of a small glass tube. The tube is filled to just above the opening of the cannula with the citrate solution. Suction is applied at the open end of the tubing and negative pressure maintained by stopping the tube with the tongue. The camera is started at high speed and the pressure is suddenly released by withdrawing the tongue from the tube. This sudden release of pressure throws the manometer into free vibrations of its own natural frequency which are recorded as seen in the figures.

TABLE 1

MANOMETER	NEEDLE		TUBE		BODY		TOTAL MASS	$\Delta Vp \times 10^{-5}$	$E' \times 10^9$	$N_{E'}$	N
	Dimensions	Mass	Dimensions	Mass	Dimensions	Mass					
1–A	5.5 × 0.074	1280	11 × 0.49	58			1338	3.9	3.4	254	250
1–B	5.5 × 0.074	1280	25.5 × 0.49	135			1415	3.9	3.4	247	240
1–C	4.5 × 0.04	3577	11 × 0.49	58			3635	3.9	3.4	154	165
1–D	4.5 × 0.04	3577	25.5 × 0.49	135			3712	3.9	3.4	152	130
2–A	2.7 × 0.074	628	22.4 × 0.305	304	2.5 × 0.5	13	945	4.2	3.1	289	280
2–B	2 × 0.04	1590	22.4 × 0.305	304	2.5 × 0.5	13	1907	4.2	3.1	204	200
2–C	8 × 0.074	1860	23.2 × 0.305	315	2.5 × 0.5	13	2188	4.2	3.1	190	200
3–A	4.2 × 0.052	1975	18 × 0.305	246	7.5 × 0.5	38	2259		About 3		Varied with different membranes above 180
3–H	8 × 0.074	1860	18 × 0.305	246	7.5 × 0.5	38	2144				
Frank Wiggers		(4, p. 18)					108 27.5		About 6.3×10^7 5.9×10^7		

In columns 2 to 7 are given the dimensions in centimeters and effective masses of the various parts of the manometer systems. In column 8 are given the total effective masses of the systems. In column 9 is ΔVp. This figure was derived experimentally by sealing a calibrated capillary pipette with sealing wax to the needle (cannula) of the manometer. The manometer was then filled so that the meniscus rose into the capillary pipette and the apparatus fixed in a pressure tight bottle so that the membrane was inside and the cannula outside. The air was sucked out to a measured pressure (dynes per sq. cm.) and Δp read directly (cc.) on the calibrated pipette. In column 10, E' is calculated from these last data by the formula $E' = \frac{\Delta p}{\Delta V}$. Columns 11 and 12 are described in the text.

Manometers 2–A, 2–B, 2–C are variations of another apparatus which likewise give good agreement between predicted and found frequencies, 3–A (for arterial use) and 3–H (for intracardiac use) designate the manometers which gave the curves appearing in this paper.

Having it in view to construct a manometer capable of following faithfully the pressure changes in the heart or artery of the unoperated animal and hoping to be able to enter these cavities repeatedly through the intact skin and without damage to the animal, we constructed a manometer to be described below. Its dimensions and constants are given in table 1, 3–A and 3–H.

The light source consisted of a 500 W. monoplane filament Mazda projection lamp inclosed in a 10-inch cubical brass box, ventilated, and with an adjustable slit in front. With the filaments fairly close to the slit, pin-

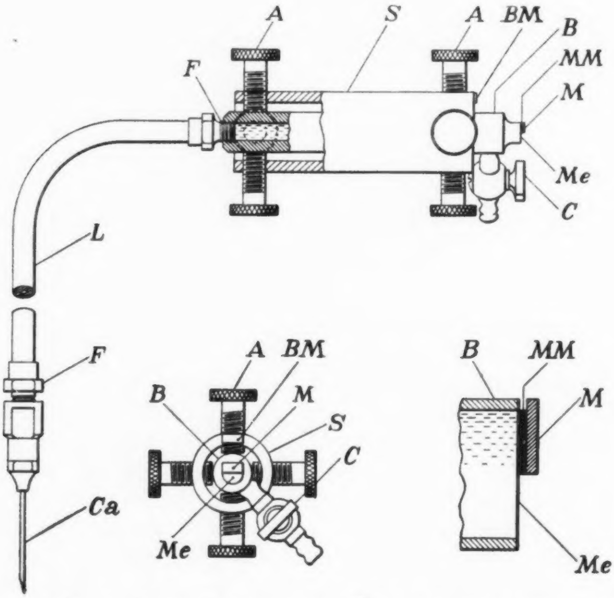


Fig. 1. Diagram of manometer. For details see text

hole images of these filaments were cast upon a horizontal row of mirrors which could record simultaneous tracings without parallax. The beam from this slit was interrupted twenty times per second by the spokes of a wheel driven by the motor of an electric clock.

The mirror (fig. 1–M) about 5 mm. square was cut from a 0.75 D plano-convex lens silvered on the flat side. These very thin lenses may be had at a nominal price as slips for bifocal spectacles from optical dealers (6).

The best mounting (fig. 1–MM) for the mirror which we have used so far is a triangular rubber cushion cemented with one point near the center and two points on the rim of the membrane. The back of the mirror is in turn cemented to this cushion.

The membrane (fig. 1–Me) consists of a flat piece of stiff shim brass 0.06 mm. in thickness. This membrane which is of sufficient diameter to cover the end of the manometer body was attached by low melting point solder so as to seal the tube; care was taken that no solder ran on to the back of the membrane.

When the membrane is attached in this fashion it is found that a few weeks' use loosens the solder. We are now working on the problem of improving the membrane attachment and mirror mounting.

The manometer body (fig. 1–B) whose wall is heavy enough to resist the strain from adjusting screws is made of phosphobronze.

The filling cock (fig. 1–C) is driven and soldered into the manometer body so that there are no projections or pockets inside.

The shell (fig. 1–S) which can be held firmly in a high-grade machined clamp forms the support for the base line mirror and for the adjusting screws. These hold the manometer body in place and enable adjustment of the beam from the membrane mirror to the beam of the base line mirror.

The base line mirror (BM) is so placed on the manometer that it serves as a control for artifacts produced by cardiac or other impacts. A straight base line in the record indicates the absence of such artifacts.

The adjusting screws (fig. 1–A) are eight in number, a set of four at each end of the shell. Their ends are rounded with a radius equal to the distance between the sets.

The screw fittings (fig. 1–F) into which the lead tubing is soldered are so constructed that there are no air pockets or obstructions inside. They are packed with soft brass washers.

The lead tube (fig. 1–L) ordinary $\frac{1}{8}$-inch commercial tubing, while adding immensely to the flexibility of the manometer and to convenience in making punctures adds relatively little to the effective mass, the greater part of which resides in the needle (cannula). The fact that the lead tube, while flexible, does not give under pressure, results in no change in the volume elasticity coefficient (E^1). The length of this tubing which we have found convenient practically is 18 cm. For cannulae (fig. 1–C) we employ ordinary Luer needles, between 16 and 22 gauge, long enough to reach the cavity to be explored. For arteries a 35° to 40° bend a centimeter or so from the end of the needle is found convenient. In any individual experiment we have selected a needle as a sort of compromise between the trauma produced by a large needle and the decrease in frequency produced by a small one. After filing the lip from the outside of the needle slip it is soldered into the brass fitting.

The long lead tube feature of this manometer makes it convenient to obtain simultaneous records without parallax from various parts of the vascular system even though they are widely separated.

Records can be made from either right or left ventricle without opening

the chest and in the thin animal if desired from the abdominal aorta by puncturing through the belly wall. In order to take records from the carotid, however, we found it necessary to expose this vessel under procaine. Unfortunately it is too elusive when approached by skin puncture.

In as much as our method implies only arterial puncture, a common clinical procedure, we expect to record human pressure pulse contours including the very rapid ones giving rise to the "pistol shot" pulse which would be beyond the scope of manometers so far used (10).

In exploring the possibilities of this manometer we have made records which are intrinsically of sufficient interest to warrant publication. They differ from records hitherto published in that they are obtained from animals which are unoperated.

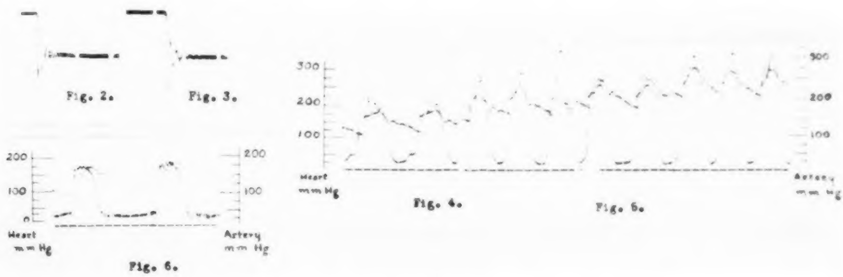
Our technique is to tie down the lightly morphinized animal and thrust the needles into those parts of the vascular system to be explored. After withdrawing the needle from the heart no untoward result is observed unless the coronary artery or a large branch has been injured. On withdrawing the needle from the carotid, the artery is held in the fingers for a few moments and then replaced in the neck under pressure. Such treatment has enabled us to use the same animal time after time. We have usually refrained from any procedure which would prevent the animal's trotting back to his cage at the completion of the experiment.

In general, blood pressures recorded in this manner are in excess of those regarded as classical for the dog (4, p. 50). This is probably due to the fact that no suspicion of shock could be entertained as regards the condition of the animal and because the heart in the unopened chest was filling normally. It will be seen from the straight base line that impacts have not distorted the record. A factor which has contributed to the controversies in the interpretation of the intraventricular pressure curve has to do with the placing of the cannula in the ventricle. In relation to this possibility in connection with our own records we have this to offer: We have taken many records which indicate clearly that the cardiac cannula became occluded by the ventricular wall during the cycle. Such records are dissimilar to those which we have shown in that systolic ventricular pressure readings are lower than those of arterial pressure. When ventricular pressures markedly exceed those of the artery we can not logically attribute this to an artifact, but only to the fact that under the circumstances of our experiment the ventricular pressure is high.

The first step in taking simultaneous records was to adjust the beams from the membrane mirrors (at 0 pressure) so that they coincided with the base line beams. The artery was then entered with the 20 gauge cannula. The long 18 gauge cannula was next thrust into the left ventricle through the chest wall and a normal record (fig. 4) taken. As seen from the cali-

bration scales the intracardiac manometer was somewhat less sensitive than the arterial manometer.

At the beginning of the cycle diastolic pressure in the left ventricle of this animal is between 20 and 25 mm. Hg. Auricular contraction raises the pressure to 50 mm. Hg in a rounded curve. We are rather skeptical of these high diastolic pressures. We have no good curves so far which fail to show them, however; but it must be kept in mind that these curves are picked from only a few experiments and do not represent our matured opinion as to the most usual curves. At the beginning of ventricular systole the pressure rises suddenly to a peak between 175 and 200 mm. Hg.



Figs. 2 to 6

Fig. 2. Frequency determination. Manometer 1-B completely filled with citrate. N = 233. Time $\frac{1}{50}$-second.

Fig. 3. Frequency determination. Air bubble in manometer 1-B. N = 90. Time $\frac{1}{50}$-second.

Fig. 4. Simultaneous pressure pulses left ventricle and carotid artery. Normal dog. Time $\frac{1}{50}$-second.

Fig. 5. Simultaneous pressure pulses left ventricle and carotid. Effect of epinephrine. Time $\frac{1}{50}$-second.

Fig. 6. Simultaneous pressure pulses left ventricle and carotid. Aortic regurgitation. High pitched musical diastolic murmur. Time $\frac{1}{50}$-second.

As seen from the next cycle this peak is not constant. We do not as yet understand its significance except that it is not instrumental. There is then a relatively slow sloping rise of pressure from 160 to 180 mm. Hg followed by a rounded peak (absent on many records) reaching a height of 200 mm. Hg. The pressure then falls rapidly during early diastole.

The rise of carotid pressure occurs about $\frac{1}{40}$ second after the beginning of ventricular systole. The diastolic value is around 100 mm. Hg and the highest systolic peak around 200 mm. Hg. Due to free vibrations in the elastic carotid (4, p. 71, 11) there is a series of two or more large oscillations followed by the incisura marking the closure of the semi-lunar valves. The mean pressure during this time is about 160 or 170 mm. Hg.

Figure 5 shows several pulses taken during the second stage of the response to epinephrine when the heart has accelerated and the minute volume is large. These show increased pressures, quick transmission time and marked accentuation of the second ventricular pressure peak. We find this type of systolic peak in many of our records and are at somewhat of a loss to explain its marked excess in pressure over the carotid. We wish again to emphasize that this cannot be due to impact or instrumental fling. An isolated chamber may be pinched off around the cannula by the contracting heart or more probably this may represent energy used to accelerate the blood stream and so would appear as velocity rather than pressure in the arterial system.

In figure 6 are shown simultaneous pressure pulses taken from the left ventricle and carotid of a dog with severe chronic aortic regurgitation. On auscultation of the precordium of this animal there was a loud high pitched musical diastolic murmur. The lower components of the murmur are recorded graphically. The manometer ($N = 200\pm$) is thrown into its own vibration frequency by the turbulence of the regurgitating blood. It is strikingly evident that a very rapid manometer is necessary to record such phenomena.

SUMMARY

The equations of Otto Frank are shown to predict the behavior of manometers whose effective masses and volume elasticity coefficients are a hundred times greater than those of manometers in common use.

Applying these equations we have constructed a manometer of adequate frequency ($N = 200$) and sensitivity which differs from the conventional optical manometer in the following respects.

1. The cannula at the end of a flexible lead tube can be inserted through the skin into the cavities of the heart or arteries.
2. Pressure pulses can be recorded from the unoperated and unanesthetized animal (or man).
3. Simultaneous records may be obtained from widely separated parts of the cardio-vascular system without parallax.

Records taken with this manometer differ from those in the literature in ways which may be interpreted as due to the absence of shock and anesthesia and due to the fact that the heart was beating normally in the unopened chest.

REFERENCES

(1) Hamilton, W. F., I. Brotman and G. Brewer. This Journal, in press.
(2) Brewer, G. W. F. Hamilton and I. Brotman. This Journal, in press.
(3) Frank, O. Tigerstedt's Handbuch der Physiologischen Methodik, 1913, II, 2 hälfte, 1.

(4) Wiggers, C. J. Pressure pulses in the cardiovascular system. 1928. Longmans, Green & Co.

(5) Hamilton, W. F., G. Brewer and I. Brotman. This Journal, 1933, **105**, 44.

(6) Hamilton, W. F. This Journal, 1930, **91**, 712.

(7) Frank, O. Zeitschr. f. Biol., 1903, **44**, 445.

(8) Wiggers, C. J. Circulation in health and disease. 1923. Lea & Febiger.

(9) Broemser, P., O. Frank and J. Petter. Zeitschr. f. Biol., 1912–13, **59**, 232.

(10) Wolf, H. J. and B. V. Bondsdorff. Zeitschr. f. d. ges. Exp. Med., 1931, **79**, 569.

(11) Broemser, P. Zeitschr. f. Biol., 1932, **93**, 149.

PRESSURE PULSE CONTOURS IN THE INTACT ANIMAL

II. Femoral Pressure Pulses in the Normal Dog and in the Dog with Aortic Regurgitation; Effect of Certain Drugs

GEORGE BREWER, W. F. HAMILTON and IRVING BROTMAN

From the Departments of Physiology and Medicine, George Washington University Medical School

Received for publication August 2, 1933

Considerable data have been obtained by the injection method (1) as to volume and velocity flow, and their regulating mechanisms, in man and in the normal dog in response to drugs and other experimental conditions (2). These responses have been contrasted with the responses of dogs with chronic aortic regurgitation (3, 4) and with cases of heart disease in man (1).

In order to amplify the picture it was decided to study the pressure changes occurring under these conditions. The methods at hand for these studies were found either inaccurate or inadequate. Estimations of the mean blood pressure (5) were inadequate to our aims. Modifications of the oscillatory indirect method and the auscultatory method as applied to the dog did not seem trustworthy. The highly accurate methods developed by Frank (6, 7) and Wiggers (8) involve major operative procedures that certainly could not be repeated every day on the same animal.

To study the pressure pulses without anesthesia or operation, i.e., under the same conditions as obtained during the volume and velocity flow measurements, we constructed a hypodermic manometer which is described in detail elsewhere (9).

With this manometer we have found it very simple to tie down the lightly morphinized dog, enter the femoral artery through the skin and take records which accurately represent changes in both systolic and diastolic pressure under conditions identical with those studied by the injection method. Furthermore, at the completion of the experiment it is possible to withdraw the needle and by a minute's pressure prevent any bleeding, thus leaving the artery in such condition that it could be punctured again and again either immediately or later.

Experimental. *The lightly morphinized dog* normally shows a femoral systolic pressure of 160 to 230, and a diastolic of from 70 to 110. These and subsequent figures apply to the normal unoperated animal and, though they appear rather high as compared with those in the literature (8, p. 50),

we feel that this is readily explained by the fact that the animals had not been widely dissected and were able upon release to trot up to their cages. The contour of the pulse is of the usual femoral type (fig. 1) with a fairly pronounced dicrotic notch.

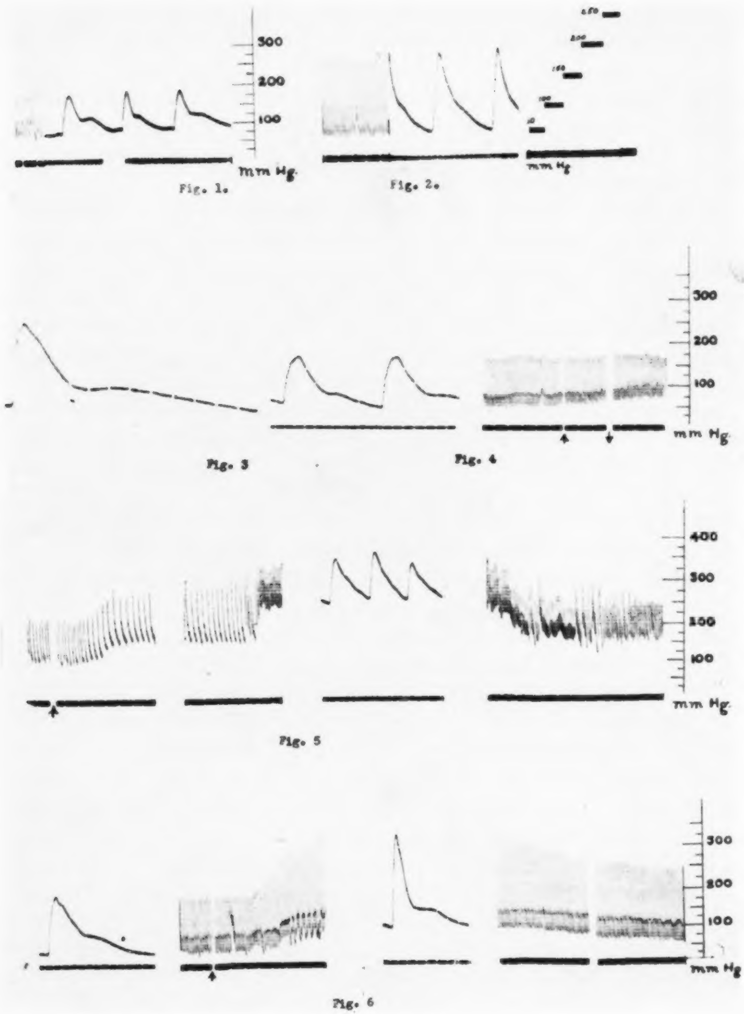
After aortic regurgitation the systolic pressure is found to range from 175 to 250 mm. Hg (see fig. 2) and the diastolic from 40 to 70. There seems to be a large variation among the different animals and even in case of the same animal at different times. In general the systolic pressure is increased and the diastolic level markedly reduced. The decided increase in the pulse pressure together with the fact that its range includes that of greater arterial elasticity than the normal pulse pressure range (10, 11), shows conclusively that the gross stroke volume must be decidedly increased. The net stroke volume is, on the other hand, decreased (3) in these dogs, and one must conclude that there is a large volume of regurgitating blood. The rate is always greater than in the normal dogs. The contour (fig. 2) has been altered from normal in two definite respects (cf. 12). The dicrotic notch is much less prominent and the systolic upstroke is much more rapid. In some of our records it is quick enough to throw the manometer ($N = 200$) into instrumental vibrations (fig. 3).

The pressure pulse curve in figure 4 differs from that in figure 3 in having a shorter quick upstroke. This dog (F, see (3)) had a somewhat larger leak than dog A (fig. 5) but much less enlargement by x-ray.

The effect of amyl nitrite is to quicken the circulation and increase the volume flow, so as to bring the dog with aortic regurgitation to within normal limits as regards these factors. We have taken many pressure records during the inhalation of amyl nitrite (5 minims) and have yet to see a definite fall in blood pressure (see fig. 4). These records were taken under the same conditions as the volume flow measurements and indicate that the unanesthetized animal regulates blood pressure by changes in volume flow to compensate vasodilatation much more accurately than does the anesthetized animal. The rate is occasionally increased and we are unable to find significant changes in pulse contour.

Acetyl-beta-methylcholine chloride, in doses of 1 to 2 mgm intramuscularly, causes the normal dog and the dog with aortic regurgitation to respond with a slight lowering of systolic pressure (about 10 mm.) and an even smaller diastolic fall (about 5 mm.). When the doses given were not so large as to cause inhibition (2 mgm. intravenously) the heart rate was increased in the normal but not in the operated dog. The remarkable changes in volume and velocity flow will be taken up elsewhere (4).

Epinephrine produces changes in the blood pressure curves which differ quite markedly as between the normal dog and the dog with experimental aortic regurgitation. In a typical normal (fig. 5), one sees evidence of the



Figs. 1 to 6

Fig. 1. Femoral pressure. Normal dog. Drum speeded up to show pulse contour. Slow record time 5 seconds. Rapid record time 0.05 second. Same for all records.

Fig. 2. Femoral pressure. Dog A with aortic regurgitation. Drum speeded to show contour.

Fig. 3. Femoral pulse of dog A with aortic regurgitation. Note instrumental vibrations from extremely rapid initial upstroke of this pulse.

Fig. 4. Femoral pulse of dog F with aortic regurgitation. Showing pulse contour and effect of inhalation of 5 M. amyl nitrite. No pressure change. No pulse rate change.

Fig. 5. Femoral pressure in normal dog. Effect of intravenous injection $\frac{1}{2}$ mgm. epinephrine.

Fig. 6. Femoral pressure in dog with aortic regurgitation. Effect of intravenous injection $\frac{1}{2}$ mgm. epinephrine.

prompt activity of the depressor mechanism which causes the heart gradually to slow (from 65 to 30 beats per minute) as the blood pressure rises. This regulation is not usually so smooth in the anesthetized animal because here one often sees the acceleration due to epinephrine interrupted jerkily by the depressor reflex during the rise in pressure. During these 20 to 60 seconds when the heart is slowed under the depressor influences, vasoconstriction serves markedly to reduce the cardiac output, per minute, and even per stroke in spite of the slow rate (2). There is also a decided increase in the bulk of blood in the heart, lungs and great vessels which reduces the velocity flow and likens the situation as a whole to that seen in cardiac decompensation.

The situation changes suddenly after about a minute, the heart accelerates (to 200 beats), and signs of depressor influences are noticeable again when the blood pressure is down nearly to normal. During this stage of the response the cardiac output has increased to well above normal and the circulation times are reduced (see 2).

When the animal with aortic regurgitation is given the same dose of epinephrine, the response is quite different (fig. 6). The heart rate accelerates within 10 to 20 seconds (from 100 to 140). There are in this record a few dropped beats which may be a weak depressor effect. Often, however, the acceleration is to about 200 with no evidence of the depressor effect, the rhythm being perfectly regular and constant. The absence of this effect when the pulse pressures are of the order of 250 to 300 mm. is very remarkable. Injections of acetyl-beta-methylcholine chloride (1 to 2 mgm. intramuscularly) before or during the epinephrine response, do not change the latter. Thus acetyl-beta-methylcholine chloride does not sensitize the vagus endings so that they may respond to whatever depressor influences may be present. Large doses (2 mgm. intravenously) of this drug, on the other hand, have a definite cardio-inhibitory action in cases of experimental aortic regurgitation. The animal with aortic regurgitation responds to epinephrine in quite the same way as the atropinized animal.

SUMMARY

1. By means of a new manometer which can be used repeatedly on the unanesthetized animal, femoral pressure pulses accurately calibrated to systolic and diastolic pressures were recorded optically.

2. The subjects investigated were normal dogs and dogs with experimental chronic aortic regurgitation. Their differential responses to epinephrine, amyl nitrite and acetyl-beta-methylcholine chloride are described. The most striking difference between the normal and operated animal is the absence of the usual depressor effects in the latter.

REFERENCES

(1) Hamilton, W. F., J. W. Moore, J. M. Kinsman and R. G. Spurling. This Journal, 1932, **99**, 534.

(2) Hamilton, W. F. This Journal, 1932, **102**, 551.

(3) Hamilton, W. F., I. Brotman and G. Brewer. This Journal, in press.

(4) Brotman, I., G. Brewer and W. F. Hamilton. Jour. Pharm. and Exper. Therap., in press.

(5) Dameshek, W. and J. Loman. This Journal, 1932, **101**, 140.

(6) Frank, O. Zeitschr. f. Biol., 1903, **44**, 445.

(7) Frank, O. Tigerstedt's Handbuch der Physioligschen Methodik, 1913, II, 2 hälfte, 1.

(8) Wiggers, C. J. The pressure pulses in the cardiovascular system. 1928. Longmans, Green & Co.

(9) Hamilton, W. F., G. Brewer and I. Brotman. This Journal, in press.

(10) Bramwell, J. C., R. J. S. McDowell and B. A. McSwiney. Proc. Roy. Soc. B., 1922–23, **94**, 450.

(11) Bramwell, J. C., A. C. Downing and A. V. Hill. Heart, 1923, **10**, 289.

(12) Brewer, G., W. F. Hamilton and I. Brotman. This Journal, in press.

FURTHER OBSERVATIONS ON FLEXOR RIGIDITY IN THE HINDLEGS OF THE SPINAL CAT[1]

J. G. DUSSER DE BARENNE AND Y. D. KOSKOFF

From the Laboratory of Neurophysiology, Yale University, New Haven, Conn.

Received for publication October 6, 1933

In a previous paper (3) the occurrence of a marked flexion posture of the hindlegs in the "secondary" decapitate or low spinal preparation of the adult male cat, i.e., after a "primary" decerebration, was described.[2] This springlike "flexor rigidity" (F.R.) is a reflex phenomenon produced by proprioceptive and exteroceptive impulses; it occurs only when the hind quarters of the animal lie prone upon the table, with the thighs abducted. This attitude is the conditio sine qua non for the appearance of the F.R., as it is absent in the side or supine position, or when the animal is held in the air. As the F.R. is often associated with prolonged priapism, during the mating periods, the syndrome was interpreted as a copulation pattern, released by the secondary decapitation or low spinal transection.

Subsequently it was found (4), however, that this same syndrome can also be elicited in the acute "primary" low spinal preparation, by putting the hind quarters of the animal in the requisite prone position upon the table.

This F.R. is found to be different in various animals. It is measured in a simple manner by noting the lengthening of a spring calibrated in grams, tied to the ankle of the animal, when the leg is passively extended by a pull on the spring. The figures observed for F.R. in the 170 experiments of this series range from 600 to 2300 grams when the animal is in the symmetrical prone position. Part of the F.R. results from mechanical

[1] Read before the annual meeting of the American Physiological Society at Cincinnati, Ohio, April 12, 1933.

[2] Griffin and Windle have mentioned in a recent paper (5), of which we have only become cognizant after the completion of our experiments, the occurrence of a flexion posture in the newborn rabbit after section of the brainstem just below the level of the nuclei of the eighth cranial nerve. Although the authors have not analyzed the phenomenon, it seems not very probable that it is related to our flexor rigidity. The brief description given suggests a different picture, and as the normal resting posture of the rabbit is a squatting one with flexion of all four limbs, it may be that the species plays a rôle in it. In the adult cat at least such flexion posture does not occur, so far as we know from the literature and our own experience, after transection of the medulla at the level mentioned above.

factors, of which the bodyweight and the stiffness of the various joints and ligaments probably are the most important; this mechanical factor in F.R., determined within one-half hour after death of the animal, ranges from 150 to 250 grams in the various animals.

This F.R. can be influenced reflexly by various procedures. If the front part of the body is lifted from the table, a marked *increase* of F.R. appears; lowering the front part of the body almost invariably results in a *decrease* of F.R. These changes are always in the same direction and usually equal in both hindlegs.

Constant and striking changes in F.R. occur when the trunk is twisted 10°–90° around its longitudinal axis by rotation of the front part of the body. These alterations of F.R. are antagonistic in the two hindlegs. The hindleg towards which the back of the animal is rotated ("back-leg") always shows a strong *increase* of F.R.; the hindleg towards which the abdomen is rotated ("abdomen-leg") always presents a marked diminution of F.R. In many instances F.R. entirely disappears from this leg and is even superseded by an active extension. The increase and decrease of F.R. on rotation of the trunk are absolutely constant phenomena and occur without noticeable latency; the active extension in the "abdomen-leg" is observed only in a large minority of the experiments and is characterized by a definite, variable latency; the longest latency observed until now was about 8 seconds. These changes in F.R. persist as long as the rotation of the trunk is maintained. The mechanical factor in F.R. is also found to be slightly increased, by 100 to 200 grams, on rotation of the front part of the body.

The active extension of the "abdomen-leg," if not present, can always be produced after injection of a non-convulsant dose of strychnine. The antagonistic reflex pattern in the two hindlegs is not changed under these conditions; this, therefore, is another instance of preservation of antagonistic, reciprocal reflex innervation under strychnine.

In a few animals the muscles involved in the production of F.R. and its changes were investigated. It was found that in the symmetrical prone position the F.R. is derived almost entirely from the contraction of the psoas, sartorius and tensor fasciae latae muscles. During the increase of F.R. on rotation of the trunk other muscles also come into play. Only after section of the whole hamstring nerve, detachment of the trochanter muscles and elimination of the adductor femoris and gracilis of one leg does the F.R. reach the values subsequently found for the mechanical factor in the other, intact leg of the animal under observation.

Passive extension of a hindleg in a spinal cat prone on the table often gives rise to distinct stretch reflexes in the flexor muscles, and to an augmentation of F.R. This can readily be seen, even through the hairy skin, as fascicular contractions in tensor fasciae latae, and on dissection in the sartorius and hamstring muscles. It might be

thought that F.R. appears only during such a passive extension; but this view is untenable, because 1, the typical, flexed posture of the hindlegs is assumed without any manipulation of the preparation; 2, passive extension of the leg in the supine or side positions does not produce F.R.; 3, passive extension of the "abdomen-leg" does not result in F.R. in this leg. Apparently these stretch reflexes in the flexor muscles, being another reflex modification of F.R., are also dependent upon the adequate, prone position of the animal.

This observation is of interest since stretch reflexes in extensor muscles of the hindlegs disappear after low spinal transection of the decerebrate preparation (1).

F.R. and its various modifications by changing the posture of the animal, as described here, are also present in the dog, immediately after spinal transection. We have been unable to observe any trace of F.R. or its changes in the acute spinal monkey.[3]

What is the source of these changes in F.R. on rotation of the trunk?

Obviously, in the low spinal preparation (transection at Th. XIII or L. I) these changes can not have their origin in tonic labyrinthine and neck reflexes (Magnus and de Kleijn), but must be caused by stimuli arising from parts of the body innervated by the severed part of the spinal cord. In the second paper (4, p. 59) these reflex changes of F.R. were considered as a new form of tonic reflexes on the flexor and extensor muscles of the hindlegs, and it was stated: "Perhaps this group of reflexes also plays a rôle as the last link of a chain of reflexes in the complex beginning with the tonic labyrinthine and neck reflexes; it (this group) can, however, as has been shown here, occur independently of these last named reflexes. It seems even as if they only become observable after isolation by spinal transection." It was then thought that stimuli arising in the muscles and joints of the caudal part of the vertebral column on its rotation might give rise to these reflex changes of F.R. This conception is incorrect, since it has been subsequently found that after bilateral section of the posterior roots of Th. X, XI, XII, L. I and II, these reflex changes in F.R., upon twisting the trunk, can be elicited just as readily and as powerfully as before the section of these roots. In this experiment all afferent impulses arising in the caudal parts of the vertebral column are eliminated; it shows conclusively that the afferent stimuli essential for these modifications of F.R. do not arise in the trunk, but must originate in parts of the body, the afferent innervation of which enters the spinal cord below L. II.

Interoceptive impulses are not essential in the elicitation of these phenomena, since they are still present after extirpation of the bladder, rectum and large intestine. The only change observed after this procedure was a decrease of the active extension in the "abdomen-leg;" this mechanism seems to be very delicate and susceptible to changes in the condition of the preparation.

---

[3] We are indebted to Dr. M. A. Kennard (of the Department of Physiology) for the information that she has not observed any evidence of F.R. in the chronic spinal monkey.

On deafferentation of one hindleg F.R. and its modifications disappear in that limb. The contralateral hindleg, with intact afferent innervation, still shows F.R. and its increase and decrease when this leg is made "back-leg" and "abdomen-leg" respectively. The active extension, however, when present in this leg before the deafferentation of the other hindleg, is no longer observable after this deafferentation. This indicates that the active extension in the "abdomen-leg" results from afferent stimuli arising in the contralateral "back-leg," and is to be regarded as a crossed extension reflex, associated with and probably resulting from the stimuli set up by the increase of the F.R. in the contralateral "back-leg." This crossed extension in the "abdomen-leg" has often been observed almost immediately after the spinal transection, as soon as the animal begins to recover from the effects of the anesthetic. In several secondary low spinal transections, in which the spinal transection could be performed without any anesthesia, as the animal was already decerebrated and had recovered from the anesthetic, this active extension in the "abdomen-leg" occurred immediately after the spinal transection.

This observation is of interest in connection with the fact that another crossed extension reflex, known as Philippson's reflex, which is present in the decerebrate cat, promptly disappears after a secondary low spinal transection. Philippson's reflex, which consists of an active extension of a hindleg on passive flexion of the other hindleg, is known also in the spinal preparation, but only in the chronic spinal animal (Sherrington), in which yet another crossed extension reflex is observable, namely, that to nociceptive stimuli applied to the contralateral foot.

The fact that the crossed extension reflex described in this paper is often observable immediately or only a few minutes after transection of the spinal cord must be attributed to the adequacy of the stimulation pattern presented. The occurrence of the reflex activities described here (F.R., its modifications by various procedures and marked stretch reflexes in flexor muscles), elicited by "natural," non-noxious exteroceptive and proprioceptive impulses in the acute spinal preparation of the cat and dog, is significant with regard to the general problem of the effects of transection of the spinal cord.

The prevalent view concerning the result of such procedure finds its latest expression in the recent monograph of Creed, Denny-Brown, Eccles, Liddell and Sherrington (2). On page 151 one finds: "Severance of the cord in the higher mammals (cat, dog, monkey) produces at once a limp quietude of the skeletal muscles aboral from the transection. . . . This muscular inactivity comprises absence of active attitude."

We also were so much imbued with this conception, that, on finding the F.R. in the "secondary" decapitate and spinal cat, it was at first assumed that this phenomenon was present in these preparations because the spinal

cord was transected only after a primary decerebration. Further experience, however, has clearly shown that this is not the case. The observations given here show that the statement quoted above is not entirely correct for the cat and the dog. Under special conditions, namely, the presentation of an adequate sensory pattern, the limp quietude of the skeletal muscles of the hind quarters is replaced by a marked "tonic" activity of the flexor muscles of the hindlegs, resulting in a definite, active, positional pattern. All depends upon the presentation of a specific sensory pattern. It is very difficult to imitate the stimuli necessary to produce F.R. Such manoeuvres as placing one's hand under the abdomen of the animal held in the air, spreading of the thighs and rubbing their ventral surface, result only in a very slight F.R. This again can only mean that a highly specific, sensory pattern is essential. Experience of this nature emphasizes the importance of a search for adequate stimulation, or better sensory patterns in attempting to establish the maximum of reflex activities of the caudal part of the spinal cord after its transection, and, it may be added, in general in studies on the activities of the central nervous system.

SUMMARY

1. Not only in the "secondary" spinal preparation of the male cat, but also in the "primary" spinal cat and dog a marked, springlike flexor rigidity (F.R.) of the hindlegs appears as soon as the animal awakes from the anesthesia, and the hind quarters are put in the symmetrical prone position on the table. During the mating periods a strong priapism is often also present. F.R. is absent in the acute and chronic spinal monkey.

2. This F.R. is a reflex phenomenon, in which exteroceptive and proprioceptive impulses, arising in the hindlegs, play a rôle.

3. F.R. is only present when the hind quarters of the animal are in contact with the table and the thighs abducted. It is absent in the side or supine position or when the animal is held in the air. If priapism is present in the prone position, it disappears in the other positions.

4. Various procedures give rise to constant changes in this F.R. Raising and lowering of the front part of the body result in increase and decrease of F.R. respectively. Torsion of the vertebral column results in an increase of F.R. in the "back-leg," and a decrease or disappearance of F.R. in the "abdomen-leg." In many instances this diminution of F.R. is even superseded by an active extension of this "abdomen-leg."

5. This active extension is another example of a crossed extension reflex. It may be present immediately after the spinal transection. After injection of a non-convulsant dose of strychnine this crossed extension reflex in the "abdomen-leg" becomes a constant phenomenon.

6. The changes of F.R. mentioned sub 4 and 5 are due to stimuli arising

in the hindlegs themselves, as they are still present after deafferentation of the caudal part of the vertebral column.

7. The fact that these reflexes can be elicited immediately after spinal transection is explained by the adequacy of the afferent stimulation pattern presented in these experiments. This point and its bearing on the symptomatology after spinal transection is briefly discussed.

REFERENCES

(1) Ballif, L., J. F. Fulton and E. G. T. Liddell. Proc. Roy Soc. London, 1925, **98** B, 589.

(2) Creed, R. S., D. Denny-Brown, J. C. Eccles, E. G. T. Liddell and C. S. Sherrington. Reflex activity of the spinal cord. Oxford Univ. Press, 1932.

(3) Dusser de Barenne, J. G. and Y. D. Koskoff. This Journal, 1932, **102,** 75.

(4) Dusser de Barenne, J. G. and Y. D. Koskoff. Pflüger's Arch., 1933, **232,** 56.

(5) Griffin and Windle. This Journal, 1931, **97,** 397.

THE VALIDITY OF THE "ALL-OR-NONE" LAW IN THE PERIPHERAL NERVOUS SYSTEM OF CRUSTACEA

T. CUNLIFFE BARNES

Contribution from the Bermuda Biological Station for Research and the Osborn Zoological Laboratory, Yale University

Received for publication August 28, 1933

Previous papers in this series on the crustacean neuromuscular mechanism have dealt with sensory impulses from movement receptors and motor impulses produced by cutting in the peripheral nerve of the crab, *Cancer pagurus* (1930a); peripheral tonus produced by prolonged and regular impulse discharges in the motor fibres (1930b); differences in the diameter of sensory fibres of the crabs *Eriphia* and *Maia* in the Mediterranean (1930c); and in the walking legs of *Cancer pagurus* and associated reflexes (1931); responses in the isolated limbs of several species of crabs in Bermuda (1932a), which are of interest in connection with the recent claim of Tonner (1933) that the muscles are influenced by axon reflexes from the skin; variation in fibre diameter in the sensory nerves of *Goniopsis* in Bermuda and sensory impulses of different sizes in *Cancer pagurus* (1932b; the oscillograph record and fig. 2 in that paper were obtained from the latter species); and finally the responses of a teratological limb of the lobster to electrical stimulation (1932c).

These studies tended to show the striking similarity between the nerve impulses of Arthropods and the better known ones of vertebrates. The existence of proprioceptive impulses from the limbs was shown, and the frequency of these impulses varied with the extent of the limb movement as in vertebrates, but the adaptation of the crustacean proprioceptors was so rapid that the term "movement receptors" was suggested. On cutting the nerve, persistent and regular series of impulses were found in motor fibres only, and it is curious that in mammals only the sensory fibres give rise to prolonged impulse discharges on cutting (Adrian, 1930). These enduring discharges set up by severing the motor fibres lasted for remarkably long periods (5 min. in several preparations of *Cancer pagurus*) and were accompanied by tonic muscular contractions. It was suggested that the persistent activity of the motor neurones might play an important part in the normal movements of the limbs but no attempt was made to correlate the length of the discharge with the strength of the single stimulus, for only cutting and traction were employed as stimuli. The proprioceptive

impulses were important evidence supporting the interpretation of geotropic orientation of Arthropods (Crozier, 1929). The motor discharges are of especial interest in connection with the neuromuscular mechanism in Arthropods in which an entire muscle is supplied with only one or two excitatory axons. The muscles are of the striated type. It is obvious that the "all-or-none" principle operates in a special way in these motor units consisting of entire muscles controlled by a single nerve fibre, and the present paper is concerned with the grading of contraction in the extensor muscle in the terminal segment of the crustacean limb and the length of the persistent series of motor impulses as a function of the strength of the stimulus (induction shock). The results are based on studies of the crabs *Cancer pagurus* and *Carcinus maenas* at Cambridge, of the *Callinectes sapidis* and *Homarus* at Yale, and especially the following interesting Decapods investigated at the Biological Station in Bermuda: *Ocypode arenarius; Callinectes ornatus;* Bermuda lobster, *Panulirus argus; Cardisoma*

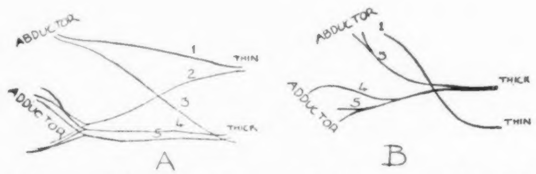


Fig. 1. A. Axons supplying the adductor and abductor muscles of the crustacean claw (according to Hoffmann, 1914a). The nerve fibres have been numbered for reference. Fibres 2 and 3 are inhibiting. The terms thin and thick refer to the two nerve strands in the limb.

B. The same scheme as found by Fraenkel-Conrat (1933). The sensory branches in Fraenkel-Conrat's diagram have been omitted.

guanhumi; Grapsus grapsus; Sesarmi Ricordi; Eupanopius Herbstii; Calappa flammea; Mithrax forceps; Goniopsus cruentatus; Microphrys bicornatus.

No complete bibliography on crustacean nerve has appeared and the problem needs an adequate review but Wiersma's (1933) recent paper summarizes most of the important work. The present paper is concerned with the "all-or-none" relations in the abductor system of the terminal segment of the limb. Hoffmann (1914) found that the leg nerve in the crayfish and lobster contains a very small number of motor fibres and his schema (fig. 1A) represents one excitatory and one inhibitory nerve fibre to the abductor which is in harmony with the observation of Biedermann (1887) and Mangold (1905) that each muscle fibre is innervated by two axons which run parallel in all the fine anastomosing nerve branches. Fraenkel-Conrat (1933) has recently questioned the existence of inhibitory fibres but his schema (fig. 1B) represents only two axons to the abductor in *Astacus*.

The phenomena of excitation in crustacea are especially interesting in connection with the "all-or-none" principle. Fortunately Ritchie (1932) has recently reviewed the present status of this law and he concludes that it represents a fundamental property of nervous tissue, in the restricted sense in which Adrian interprets the principle. Adrian (1932, p. 62) writes: "It would be wiser I believe to say, not that muscle and nerve obey the principle, but that in both of them there is an 'all-or-nothing' relation between the stimulus and the propagated disturbance." Ritchie's interpretation of the recent interesting work concerning the alleged grading in single muscle fibres in vertebrates (cf. Fischel and Kahn, 1928; Hintner 1930; Pratt, 1930; Gelfan, 1930) has been confirmed by Gelfan and Bishop (1932) who have shown that there is no action potential during a submaximal contraction in a single muscle fibre of the frog's retrolingual membrane. In other words these apparent exceptions of the "all-or-none" law in muscle fibres do not involve the propagated disturbance. Ritchie considered the possibility that the nerves of invertebrates may not exhibit an "all-or-none" relation and this must be granted, for example, in the primitive nerve net or in the pseudopodium of amoeba; but the experiments reported in the present paper indicate that the "all-or-none" principle has a wider scope than hithertofore supposed and holds in the peripheral nerves of Arthropods. As Adrian (1932) remarks, the presence of only a very few motor axons can be understood in the case of insect muscles with few fibres, but it is a remarkable fact that the relatively large muscles of crustacea can be governed by one or two motor axons. The regular rhythms recorded in these fibres (fig. 6) show that the "all-or-none" law is certainly valid in the nerve, but the possibility remains that the crustacean muscle fibre may be capable of gradation.

Methods. Oscillograph records previously made through the courtesy of Prof. E. D. Adrian (figs. 5 and 6) were studied in connection with records of the muscular contraction in the species of Bermuda decapods mentioned above. The excised limbs and claws were stimulated with platinum or Ag-AgCl electrodes by means of a Harvard inductorium (2 dry cells). In all cases the flexor tendon of the terminal segment (dactylus) was cut, leaving the abductor supplied with a single excitatory axon (according to Hoffmann's scheme). Natural articulation of the dactylus was retained, the tip moving over a millimetre scale. In other cases the flexor tendon was cut on the intact animal and the extension of the digit observed under reflex stimulation.

The motor discharges set up in the nerve by single induction shocks were followed by an amplifier and a loud speaker. A modification of the amplifier previously used (Barnes, 1932a) was adopted, consisting of 6 high mu (= 30) valves, the first three having their filament voltage halved and adjusted through a rheostat. In this way the system could be stabilized.

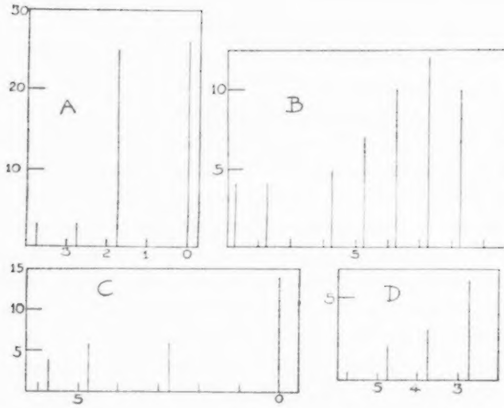


Fig. 2. Extension of the dactylus as a function of the strength of induction shocks (Harvard coil and two dry cells). Abscissae: distance of secondary coil. Ordinates: movement of the tip of the extending digit in millimeters. The flexor tendon was cut and the nerve stimulated in the merus by make-break contacts at about 5 per second. The final maximum extension is indicated on the graphs. In figure 3A and B the number of stimuli is shown.

A. (No. 102.) Bermuda lobster, *Panulirus argus*, leg. 15 July T. 25°C.
B. (No. 95.) Ghost crab, *Ocypode arenarius*, leg. 12 July.
C. (No. 108.) Land crab, *Cardisoma guanhumi*, leg. 18 July T. 27.2°C.
D. (No. 115.) *Sesarmi Ricordi*, leg. 21 July.

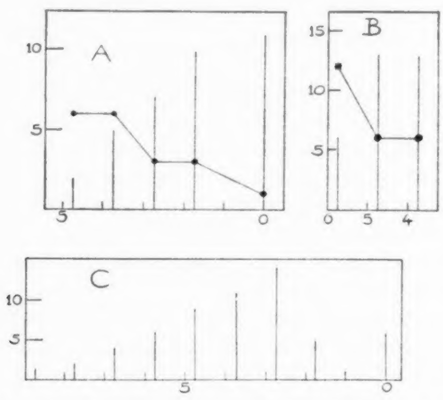


Fig. 3. Abscissae and ordinates as in figure 2 but the black dots indicate the number of stimulations (at about 5 per sec.).

A. (No. 82.) *Callinectes ornatus*, claw.
B. (No. 115.) *Ocypode arenarius*, leg.
C. (No. 83.) *Callinectes ornatus*, leg.

Isolated limbs. A more or less pronounced grading was obtained in the extensor of the dactylus of all the forms investigated (figs. 2, 3 and 4). Of about 90 preparations we have selected the ones represented in the figures. In all these the flexor tendon was cut and the excitation reached the abductor *via* the nerve. On Hoffmann's (1914) interpretation there was only one excitatory axon to the muscle whose contraction was measured. It is therefore of great interest to observe the graded control of the muscle in the absence of hundreds of motor axons as in vertebrates. In some preparations (i.e., no. 102, walking leg of the Bermuda lobster, fig. 2A)

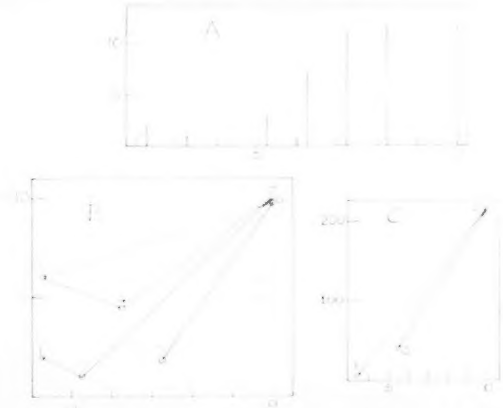


Fig. 4. A. (No. 73.) Box crab *Calappa flammea*, leg. 7 July T. 27°C.

B and C. Duration of persistent rhythm of motor impulses in merus nerve of the common lobster (*Homarus*), New Haven.

Abscissae: secondary coil distance as in other figures.

Ordinates: duration of nervous discharge in seconds. The stimulus in each case was a single contact (make-break).

B. Thin nerve in walking leg. 8 May T. 21.5°C. The numbers indicate the order in which the volleys were produced.

C. Thin nerve in walking leg. 5 Nov. T. 19.8°C.

the contraction approaches a gross "all-or-none" effect, while in others (walking limb of *Callinectes*, no. 83, fig. 3, and *Ocypode*, no. 95, fig. 2) a delicate grading is readily seen. The total contraction often occurs in steps following each induction shock (about 5 per sec.) but in figure 3A and B it will be observed that the grading was not produced by increasing the number of the stimuli since, with stronger currents, a single shock will often elicit greater extension than repeated stimulation at a greater secondary coil distance. That all the grading was not due to facilitation by previous activity was shown by decreasing the strength of each successive stimulus. At strong shocks (see fig. 3C) the contractions sometimes diminished, probably because of injury.

Reflex stimulation. In some intact animals the flexor tendon of the dactylus on one of the limbs was cut, and the graded movements of the extensor observed under reflex stimulation. Specimen 58, *Grapsus grapsus*, (1 July, T 27°C.) is an example. Flexion of all the limbs was produced by tapping the ventral surface of the animal but no flexion of the operated dactylus occurred indicating that only the extensor remained functional.

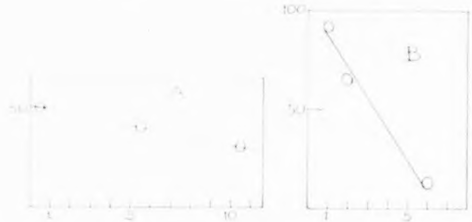


Fig. 5. Decline in frequency of regular series of impulses in the persistent rhythm set up in crab's nerve by cutting. (Taken from oscillograph records cf. Barnes 1930a.)
Abscissae: duration of rhythm in seconds.
Ordinates: impulses per second.
A. Shore crab *Carcinas maenas* (caught at Yarmouth, England).
B. Edible crab *Cancer pagurus*, Cambridge.

Fig. 6. Oscillograph records of a persistent discharge in a single motor fibre in the meropodite nerve of the crab *Cancer pagurus* (cf. Barnes, 1930 b). The two sections of the record are taken from the beginning and end of the discharge, to show the remarkable regularity of the rhythm. These regular series obtained from the entire nerve across the electrodes, support Hoffmann (1914a), that very few motor axons are present. Record obtained at Cambridge through the kindness of Prof. E. D. Adrian.

After moving the limb into a flexed position the operated dactylus extended 1.5 cm. after which "spontaneous" extensions of various extent were observed (the first one measured being 5 mm.). A better example was *Cardisoma* (no. 124) in which the following extensions of the operated dactylus were measured (under reflex stimulation) in the order given: 3 mm.; 2 mm.; 1 mm.; 1 mm.; 3 mm.; 1 mm.; 4 mm.; 4 mm.; 10 mm.; 5 mm.; 12 mm.; 4 mm.; thus giving a beautifully graded series. These preparations (typical of several others of other species) show that the grading effect in

this motor unit was not an artifact conditioned by electrical stimulation. Hoffman (1914b) has made the same observation in the crayfish.

Nervous discharges. Volleys of impulses in the motor fibres were followed with an amplifier and loud speaker. The regularity of these series indicated that a very few single fibres were in action, as is also seen in oscillograph records previously made through the kindness of Prof. E. D. Adrian (figs. 5 and 6). These records obtained by cutting the entire nerve in the

TABLE 1

Duration of discharges of motor impulses in the nerve of Homarus as controlled by the strength of the initiating induction shock

Only the thin nerve was used, which (according to Hoffmann (1914a)) contains the excitatory axon to the abductor of the dactylus.

NUMBER	DATE	TEMPERATURE	SECONDARY COIL DISTANCES	DURATION OF VOLLEY
		°C.	cm.	sec.
133	1 Nov. '32	22	$5\frac{3}{4}$	1
			$2\frac{3}{4}$	4
			$5\frac{3}{4}$	$\frac{1}{2}$
			$1\frac{3}{4}$	1
134	5 Nov. '32	19.8	$6\frac{3}{4}$	6
			0	215
			$4\frac{3}{4}$	40
136 a	9 Nov. '32	20	$7\frac{3}{4}$	15
			3	32
			0	10
144	8 May '33	21.5	$5\frac{3}{4}$	2
			$4\frac{3}{4}$	1
			0	10
			$3\frac{3}{4}$	4.2
			$5\frac{3}{4}$	6
			0	9.4
			$2\frac{3}{4}$	1.8
			0	0

crab's limb occasionally show remarkably constant frequency (fig. 6) but usually the frequency declines (fig. 5) after the stimulus, and the impulses cease after a few seconds to several minutes. We have since made a special study of the duration of the series of impulses as influenced by the strength of an induction shock which will also initiate the discharge. It is found that the stronger the single shock, the longer the discharge lasts. (Figs. 4B and C and table 1.) The duration of the volley was determined by means of a loud speaker. The lobster (Homarus) was selected as the best material for this investigation because the excitatory fibre to the

abductor can be isolated readily (it runs in the thin nerve). Some preparations (i.e., no. 133) gave nervous discharges of relatively short duration ($\frac{1}{2}$ to 4 sec.) but the dependence on strength of stimulus was nevertheless evident. At the other extreme one may mention no. 134 in which the strongest shock set up an impulse volley which lasted as long as 215 seconds but this was reduced to 6 seconds with weak stimulation (secondary coil distance of $6\frac{3}{4}$ cm.). The readiness with which these unmedullated fibres became fatigued makes it difficult to repeat a series of stimulations, but in preparation 144 a discharge of 10 seconds was obtained at full strength and after shorter volleys obtained with weaker shocks, a duration of 9.4 seconds was again secured with the coil pushed home. In connection with the measurements of muscular contraction which seemed to yield an "all-or-none" effect, one may mention no. 141 (8 May, T. 21.5°C.) which gave no detectable discharge at secondary coil distances of $6\frac{3}{4}$ and $4\frac{3}{4}$ cm. but at 0 cm. a discharge of 38 seconds' duration was obtained, accompanied by marked extension of the dactylus. That the degree of contraction is at least partially dependent on the *duration* of the discharge may be inferred from preparation 133 in which the following correlations were found: at a secondary coil distance of $5\frac{3}{4}$ cm. the duration was 1 second and the dactylus extended 2 mm., while at $2\frac{3}{4}$ cm. the duration was 4 seconds and the extent of the contraction 20 mm. From analogy with vertebrate muscle (cf. Adrian, 1932) it is evident that the frequency of the nervous impulses must also play an important rôle, but in motor units exhibiting such pronounced heterochronism a new factor, i.e., the *duration* of the nervous discharge seems to be equally important. In preparations of *Cancer pagurus* in which the frequency of motor impulses after cutting was determined with an oscillograph (fig. 5) it was observed that the extent of contraction of the muscles was determined by frequency as well as by the duration of the discharge. Immediately after cutting the nerve, when the frequency of regular series was 100 per second, closing of the claw occurred which was immediately followed by opening (extension) of considerably longer duration, and the extent of the contraction fell *pari passu* with the declining frequency of the motor impulses. The persistent discharges are characteristic of neuromuscular mechanisms exhibiting pronounced heterochronism (cf. Lapicque, 1912; Jasper and Monnier, 1933).

Discussion. The "all-or-none" law represents a fundamental mechanism of rhythmicity (refractory period, threshold and complete discharge) in irritable tissues and the above considerations of previous work and new data concerning crustacean nerve, demonstrate that the principle applies to the nerves of Arthropods. It has been believed hitherto (cf. Jordan, 1928) that the principle does not apply to the nerves of invertebrates but the remarkably regular rhythms (cf. fig. 6) typical of crustacean nerve could not occur if the principle did not apply. There is no indication in

our oscillograph records or those of Monnier and Dubuisson (1931) that the size of the impulse depends on the strength of the stimulus on the same fibre as Du Buy and Reitsma (1928) and Jordan (1928) suppose. The two waves obtained by Monnier and Dubuisson are evidently conveyed by motor fibres of different diameter (it is known that the motor fibres are not of the same calibre) and are probably associated with the rapid and slow contraction of the adductor. Also there is sufficient variation in diameter in the sensory fibres (Barnes, 1932b) to account for impulses of different size in the sensory fibres; in fact Jasper (1933b) found the slow wave upon sensory stimulation. Du Buy and Reitsma (1928) base their conclusion that the impulse is not of an "all-or-none" character on experiments in which "block" occurred more readily if two points on the nerve were narcotized, but the nerve was not removed from the limb, and hence the localization of the narcotized region (wad of cotton with cocaine) can not have been very exact. The pronounced grading in crustacean muscle is not produced by varying the size of the impulse in the nerve fibres, but seems to depend on some nervous mechanism quite compatible with the "all-or-none" principle. In a recent paper Wiersma (1933) considers the quick contraction as "all-or-none" but not the longer contractions for which he considers a summation mechanism.

It is not known how the gradation in the contraction of the muscle fibres is controlled but the small number of excitatory nerve fibres precludes the possibility of multifibre summation and the only nervous phenomena which vary with strength of stimulus are *frequency* and *duration* of impulse discharge. It is suggested that a second (or more) contraction may occur in a single fibre before the first has subsided. This is supported by a number of considerations: 1, crustacean muscle is capable of extensive summation; 2, a marked heterochronism obtains; 3, the axons give persistent discharges; 4, the economy of maintained contractions (Bronk, 1932) is achieved through a slowing of the muscle due to previous activity which favours a summation mechanism.

On the humoral theory of nervous action, the summation might well depend on the amount of a substance whose production depends on the number of impulses arriving in unit time. Indeed, the phenomena of "rebound," "inhibition," "augmentation" and after effects seen in these peripheral preparations (Knowlton and Campbell, 1929) are usually explained on a chemical basis. Moreover, Fredericque (1928) and Perkins (1928) have described humoral effects in crustacean nerve, and its high metabolism (Hill, 1932) is quite suggestive.

We have studied the gradation in the abductor, since it possesses the smaller number of axons. In most of our preparations the second (inhibitory) fibre was intact, but it responds to a different current strength and it is probable that the effects were conditioned only by the single excitatory

axon. Moreover, in the lobster we eliminated this inhibitory fibre by isolating the thin nerve. In any event the large number of gradations exceeded the possible number of motor neurones.

The second axon to the abductor running in the thin nerve with the adductor fibres (Hoffmann, 1914a) and possessing the same threshold as these, is usually regarded as an inhibitory fibre. We do not propose to review the theory of specific inhibitory axons in Arthropods, but obviously it must be kept in mind that Fröhlich (1908) and Fraenkel-Conrat (1933) deny the existence of purely inhibitory fibres which would mean that in most of our preparations there were two excitatory fibres; but in any event the number of contraction steps far exceeded the possible number of nerve fibres. On the other hand, the discovery of Verzar and Ludany (1929) of action currents in the inhibited antagonistic muscle, supports the original theory of Biedermann that inhibition in Arthropods is an active process requiring specific axons and not merely "absence of stimulation" as in vertebrates. Also the phenomena of arrest, rebound and rhythmical contraction so characteristic of these peripheral preparations strongly suggests that inhibitory fibres are present. The fact that the "all-or-none" law holds in the nerve, makes possible Wedensky inhibition (which Fraenkel-Conrat, 1933, believes is the only type of inhibition occurring in the peripheral nerves of crustacea); but this explanation is only possible with strong currents and high frequencies, while the inhibition of the adductor occurs at low frequencies and weak stimulation. Even if it be granted that purely inhibitory axons were present, it must be remembered that Segaar (1929) has interpreted his results on the grounds that the properties of the peripheral nerves are determined by the state of activity of the ganglia and the same axon can inhibit at one time and excite at another. Auger and Fessard (1933b) regard the rhythms in crustacean nerve as a manifestation of a general property of protoplasm seen more clearly in ganglia (Adrian, 1932b).

Perhaps even more striking than the special way in which the "all-or-none" law operates is the central character of the nervous effects obtained in these peripheral preparations and as Hill (1929 p. 175) remarks, it is not improbable that crustacean nerve muscle preparation will make it possible to investigate a number of the more inacessible properties of the central nervous system of vertebrates.

SUMMARY

1. The validity of the "all-or-none" law in the crustacean nerve muscle preparation is considered in the light of previous work and of additional data obtained with Bermuda crustacea.

2. It is concluded that the "all-or-none" principle as interpreted by

Adrian holds in crustacean nerve as a basis for the remarkable rhythms characteristic of the motor axons.

3. The duration of these rhythms was found to be a function of the strength of the initiating induction shock.

4. Pronounced grading in the contraction of the abductor muscle of the dactylus was produced by stimulating nerve with single induction shocks of graded strength.

5. The steps in the gradation of muscular contraction were far more numerous than the number of motor axons supplying the muscle, indicating that the "all-or-none" principle operates in a special way.

6. It is suggested that crustacean muscle fibre is capable of extensive summation controlled by the *duration* as well as the frequency of the impulse discharge in the motor axon.

I am greatly indebted for the indispensable technical assistance of Miss A. K. Cunliffe Barnes. It is also a pleasure to express my thanks to Dr. J. F. G. Wheeler who placed the facilities of the Bermuda Station at my disposal. The work was made possible by a grant from the Osborn Zoological Laboratory, Yale University.

REFERENCES

Adrain, E. D. 1930. Proc. Roy. Soc., B, **106**, 596.
1932a. The mechanism of nervous action. Philadelphia.
1932b. Journ. Physiol., **72**, 132.

Auger, D. and A. Fessard. 1928. Compt. Rend., **99**, 305.
1933a. Ibid., **112**, 863.
1933b. Ibid., **113**, 1441.

Barnes, T. C. 1930a. Journ. Physiol., **69**, Proc. 32.
1930b. Ibid., **70**, Proc. 22.
1930c. Compt. Rend., **105**, 385.
1931. Journ. Physiol., **71**, Proc. 12.
1932a. This Journal, **99**, 321.
1932b. Ibid., **100**, 481.
1932c. Zeitschr. verg. Physiol., **18**, 280.

Biedermann, W. 1887. Sitz. ber. k. Akad. d. Wiss., **96**. 1895. Elekrophysiologie. 1898. Transl. by F. A. Welby.

Blaschko, H., M. Cattell and J. L. Kahn. 1931. Journ. Physiol., **73**, 25.

Bronk, D. W. 1932. Journ. Cell. and Comp. Physiol., **2**, 285.

Chauchard, A. B. and P. Chauchard. 1932a. Compt. Rend., **113**, 136.
1932b. Ibid., **113**, 621.

Chang, T. H. 1931. Proc. Soc. Exp. Biol. Med., **28**, 954.

Crozier, W. J. 1929. The study of living organisms. Chap. 2 *in* Murchison's foundations of experimental psychology. Worcester.

Ewald, W. F. 1911. Arch. f. Physiol., **181**.

Fischl and Kahn. 1928. Pflüger's Arch., **219**, 33.

Fraenkel-Conrat, H. 1933. Zeitschr. verg. Physiol., **19**, 38.

Fredericq, H. 1928. Arch. Intl. Physiol., **30**, 311.

Fredericq, L. and G. Vandevelde. 1879. Bull. Acad. Roy. Belg. Ser. 2, **97,** 6.
Frohlich, F. W. 1908. Zeitschr. f. allg. Physiol., **7,** 393.
Furusawa, K. 1928. Journ. Physiol., **65,** 28.
1929. Ibid., **67,** 325.
Gelfan, S. 1930. This Journal, **93,** 1.
Gelfan, S. and G. H. Bishop. 1932. This Journal, **101,** 678.
Hill, A. V. 1929. Proc. Roy. Soc. B, **105,** 153.
1932. Chemical wave transmission in nerve. New York.
Hintner. 1930. Pflüger's Arch., **224,** 608.
Hoffmann, P. 1914a. Zeitschr. Biol., **63,** 414. 1914b. Ibid., **64,** 247.
Jasper, H. 1933a. Compt. Rend., **112,** 230.
1933b. Biol. Bull. **65,** 371.
Jasper, H. and A. M. Monnier. 1933. Compt. Rend., **112,** 233.
Jordan, H. J. 1928. Arch. néerland. Physiol., **13,** 570.
Knowlton, F. P. and C. J. Campbell. 1929. This Journal, **91,** 19.
Lapicque, L. and M. Lapicque. 1910. Compt. Rend., **69,** 278.
Lapique, L. 1912. Ibid., **155,** 70.
Levin, A. 1927. Journ. Physiol., **63,** 113.
Luchsinger, B. 1882. Pflüger's Arch., **28,** 60.
Lucas, K. 1907. Journ. Physiol., **35,** 310.
1917. Ibid., **51,** 1.
Mangold, E. 1905. Zeitschr. allg. Physiol., **5,** 135.
Monnier, A. M. and M. Dubuisson. 1931. Arch. Intl. Physiol., **34,** 25.
Perkins, E. B. 1928. Journ. Exp. Zool., **50,** 71.
Pantin, C. F. A. 1929. Journ. Physiol., **67,** Proc. **44.**
Piotrowsky, G. 1893. Ibid., **14,** 163.
Pratt, F. H. 1930. This Journal, **93,** 9.
Reitsma, A. and H. G. Du Buy. 1928. Tidjschrift Ned. Dierk. Ver. 3C. Serie Dl. i, Afl. 2, 55.
Richet, C. 1882. Physiologie des muscles et des nerfs. Paris.
Ritchie, A. D. 1932. Biol. Reviews, **7,** 336.
Segaar, J. 1928. Kononklyte Akademie von Wetenschappen te Amsterdam Proc. **31,** no. 8, 15.
1929. Arch néerl. Physiol., **14,** 241.
1929. Zeitschr. verg. Physiol., **10,** 120.
Tonner, F. 1933. Zool. Jahrb., **53,** 101.
Uexküll, J. Von und F. Gross. 1913. Zeitschr. Biol., **60,** 334.
Verźar, F. und G. Von Ludany. 1929. A Magyar Biologiai Kutato Intezet Munkai., **2,** 243.
Wiersma, C. A. G. 1930. Tidj. Ned. Dierk Ver. 3 Ser. D. ii, Afl. 2.
1933. Zeitschr. ver. Physiol., **19,** 349.

THE EFFECT OF A DEFICIENCY OF VITAMIN B[1] UPON THE CENTRAL AND PERIPHERAL NERVOUS SYSTEMS OF THE RAT

C. O. PRICKETT[2]

From the Laboratory of Animal Nutrition, Alabama Polytechnic Institute, Auburn, Alabama

Received for publication October 6, 1933

Numerous studies of peripheral nerves from vitamin B-deficient animals have been reported (Eijkman, 1897; Voegtlin and Lake, 1919; McCarrison, 1921; Findlay, 1921; Cully, 1927; Vedder and Clark, 1927; Moore, Brodie, and Hope, 1927; Woollard, 1927; Stern and Findlay, 1929; Zimmerman and Burack, 1932). For the most part, the tissues used in these studies have been prepared by the Marchi method or by one of its modifications. The preponderance of evidence has indicated that myelin damage is associated with a deficiency of vitamin B, but whether the symptoms observed in vitamin B-deficient animals can be explained on the basis of such damage is a moot question (Kruse and McCollum, 1933; Harris, 1932, 1933). The uncertainties in the interpretations of Marchi preparations which have been emphasized by Duncan (1931) even suggest that myelin damage in the peripheral nerves of vitamin B-deficient animals has not been conclusively demonstrated.

Studies of the central nervous system from animals whose diets contained an inadequate supply of vitamin B have been less numerous. Pappenheimer and Goettsch (1931) found edema, necrosis, and hemorrhages in the cerebellum of chicks on a diet low in vitamin B; Kingery and Kingery (1925) observed consistent and characteristic changes in the Purkinje cells of the cerebellum, in motor cells of the spinal cord, and in the cells of the spinal and sympathetic ganglia of rats fed a diet deficient in vitamins A and B; Zimmerman and Burack (1932), working with dogs on a diet deficient in the vitamin B complex, concluded that "ganglion cell changes and disseminated foci of myelin destruction in the brain or spinal cord could not be held responsible for the clinical symptoms of this nutritional disorder."

[1] Vitamin B refers to the heat-labile fraction and vitamin G to the heat stable fraction as designated by the Committee on Nomenclature of American Society of Biological Chemists (1929).

[2] The author wishes to thank Prof. W. D. Salmon for constant aid during this investigation, and J. G. Goodman for the care of the animals.

The literature in the past has been concerned almost entirely with changes observed in the tissues of animals whose diets were deficient in the vitamin B complex or, in some cases, consisted of polished rice alone. It is the purpose of this paper to report studies of the nervous tissues of animals whose diets were free from the heat-labile fraction, vitamin B, but contained an adequate amount of heat-stable fraction, vitamin G.

EXPERIMENTAL: METHODS. *Grouping of animals.* The general plan adopted for this experiment was to place young rats averaging 40 to 50 grams in weight on a diet which was free from vitamin B but adequate in all other respects. The animals were allowed to continue on this diet until they developed the severe symptoms characteristic of the deficiency, were then chloroformed for necropsy, and the tissues prepared for microscopic study. These animals are referred to as the deficient group. This group

TABLE 1

Composition of basal diets

INGREDIENTS	DIET 552	DIET 553
	grams	*grams*
Casein (purified)	18.0	18.0
Salt 186*	4.6	4.6
Agar	1.0	1.0
Sucrose	43.4	43.4
Butter fat (filtered)	23.0	
Cod liver oil	2.0	2.0
Autoclaved yeast**	8.0	8.0
Cocoanut oil		23.0

* Salt mixture 186; W. D. Salmon, Journ. Biol. Chem., 1930, **89**, 200.

** Northwestern dried yeast, autoclaved 8 hours at 17 pounds pressure.

consisted of 126 rats of which 57 were on diet 552, 17 on diet 553, and 52 on miscellaneous diets, all of which were deficient in vitamin B but differed from diet 552 in the type and amount of fat. The composition of diets 552 and 553 is shown in table 1.

A few rats in the deficient group were allowed to develop severe symptoms, given a curative dose of vitamin B concentrate, and then allowed to continue on the deficient diet until the second onset of spastic symptoms. This group will be referred to as the recurrent group.

The experiment also included three control groups. Group A, consisting of 14 rats, received diet 552 and in addition an adequate amount of vitamin B concentrate. The food consumption of this and the deficient group was controlled by the paired feeding method. Group B, consisting of 11 rats, received diet 552 and in addition the same amount of vitamin B concentrate as received by group A. The animals in this group received

food ad libitum. Group C, consisting of 24 normal young animals from the stock colony, were fed the stock ration (table 2) and were sacrificed at approximately the same age as the rats in the deficient group and control groups A and B.

Necropsy. Animals in the deficient group were allowed to develop severe symptoms and were left until moribund or as close to this point as deemed advisable. In so far as possible, one animal from each of the control groups was killed at the same time. In no case was necropsy performed more than fifteen minutes after death. At necropsy complete gross observations were made of all organs; however, as this study is primarily concerned with the nervous tissues, only passing comment will be made of other changes noted.

TABLE 2
Composition of stock diet

INGREDIENTS	GRAMS
Casein (com.)	10.0
Ground wheat	60.0
Meat scraps	5.0
Tankage	5.0
Linseed meal	4.0
Alfalfa meal	2.0
Bone meal	2.0
Iodized salt	1.0
Molasses	5.0
Butter	5.0
Cod liver oil	1.0
Whole milk	daily

Great care was taken in the removal of the peripheral nerves (sciatics and brachial plexuses) to be studied. The whole extremity, after removal of the skin and upper layers of muscle to bare the nerve so that rapid fixation could take place, was immersed in the desired fixative. In only a few cases, in which it was desired to place portions of the same nerve in different fixatives, were the nerves sectioned or disturbed from their natural position until fixation was complete. In most cases the paired nerves were prepared by the same technic, but in some cases the right nerve was prepared by one technic while the left nerve was prepared by a different technic. It was hoped in this way that variations due to technic could be more readily observed.

Histological technic. The nervous tissues of both the deficient and the control groups were fixed in either 95 per cent alcohol, 10 per cent formalin (U.S.P. 1:10), or in 3 per cent potassium dichromate. The tissues fixed in 95 per cent alcohol were used for the Nissl and hematoxylin-eosin tech-

nics; those fixed in 10 per cent formalin were used for the Busch, formalin-Marchi,[3] sudan III, and Spielmeyer technics; those fixed in 3 per cent potassium dichromate were used for the Marchi technic. Care was taken to keep the concentration and volume of the osmic acid solutions as well as the time the tissues were in the solutions uniform to avoid, in so far as possible, any variations due to procedures. In the case of the Busch and formalin-Marchi, the formalin was thoroughly washed out before refixation in 3 per cent potassium dichromate. The Nissl material was stained with either toluidin blue or thionin.

The material was embedded in either celloidin or gelatin or was unembedded. No differences due to the type of embedding could be noticed in sections of material prepared by the osmic acid methods, but as a precaution the tissue embedded in celloidin was left in the various celloidin solutions the minimum time necessary for good penetration.

RESULTS. *Symptomatology.* The animals in the deficient group usually showed a considerable gain in weight during the first week, which was succeeded in the second week by either a slight gain or a slight loss in weight. From this time onward, there were usually a consistent loss in weight and a progressive degree of excitability. The most characteristic and consistent symptoms, however, manifested themselves about the 28th to the 40th day. The most marked of these was an incoördination in the movements of the posterior extremities which resulted in the development of a halting, swaying gait. Involvement of the anterior extremities by this incoördination was less apparent. The incoördination became more marked as the experiment went on and usually resulted in inability to walk before the animal became moribund. The head was usually involved as shown by a tendency to incline it to one side or to retract it markedly. Palsied shaking or bobbing of the head was often seen. In the later stages the animal appeared to be blind. The eyes protruded and had a glassy, staring look. If the vibrissae were clipped the animal would run about bumping against the feed jar and sides of the cage or, if placed on a table, would run off the edge. A pencil pointed at the eye produced no reaction until the cornea was touched.

Intermittent convulsive seizures and extreme spasticity characterized the later stages of this condition. All animals included in this study developed marked symptoms, although the time of onset, the severity of the symptoms, and the length of life after development of the symptoms varied somewhat with individuals and with the type and amount of fat in the diet. Growth of animals in control group A approximated that of animals in the deficient group. The animals in control group B were slightly lighter in

[3] Fixation in 10 per cent formalin with subsequent washing, refixation in 3 per cent potassium dichromate and staining in Marchi fluid.

weight at the time of death than animals in control group C, but the difference was so small as to appear insignificant.

Gross findings. Emaciation, decrease in size of all organs, mild enteritis, and generalized congestion were common to the animals in the deficient group and control group A, while control groups B and C showed no significant changes at any time. In both the deficient group and control group A an atrophy of the thymus was observed; in the deficient group, the thymus at the time of death was reduced to a few shreds of fat-like material, whereas, in control group A, although the organ was very much decreased in size, it kept its normal shape and consistency.

Striking changes were observed in the pons, medulla, or cerebellum in approximately 75 per cent of the animals in the deficient group. These changes consisted of disseminated foci of hemorrhage or marked congestion of one or both sides. The hemorrhage varied in size from a pin point to large areas 1 to 2 mm. in diameter (fig. 1). These hemorrhages were usually bilaterally symmetrical, but in a few cases occurred on only one side while the opposite side showed marked congestion. The site of the hemorrhage was usually in the floor of the fourth ventricle at the level of the eighth nerve in the general region of the vestibular and cochlear nuclei; however, the medulla of the cerebellum was quite frequently involved, and in two cases, foci were observed in the region of the superior olivary nucleus. Animals in the recurrent group usually showed an intensification of the lesions in these areas. In some individuals in this group fresh hemorrhages were seen to intermingle with older ones. In animals not showing frank hemorrhage, marked congestion was observed in these areas. None of the animals in the control groups evidenced at any time congestion or hemorrhage in this or any other region of the brain except those animals in control group A, in which a generalized congestion was observed.

Microscopic findings in the peripheral nervous system. Marchi preparations of peripheral nerves showed a variable amount of diffuse black precipitate in the deficient and in all the control groups (fig. 2). No significant difference could be noted in either the amount or distribution of this precipitate in the various groups. There appeared to be a slight degree of foaminess, and some irregularity in size of the neurons, especially at the nodes of Ranvier, but as this was also found in the control groups it was considered of no significance. The precipitate consisted of flaky masses, vacuolated masses, balls, and rods which were found as often within as without the neurilemma (fig. 3).

Perineural fat, when present, was intensely stained. At times fibers showing changes typical of Wallerian degeneration were seen, but these were infrequent and were found in all groups. No apparent difference was observed between the amount of blackening in proximal and distal portions of the nerves. The sciatics contained no more black precipitate than

did the brachial plexuses. In cross section many of the fibers evidenced ringlets of blackened material about the axons. In the deficient group no apparent relationship could be found between the amount of diffuse blackening and the severity or duration of the symptoms.

Nerves prepared by the formalin-Marchi method evidenced the same type and distribution of blackened material in both the deficient and control groups, but as compared with the Marchi preparations the amount appeared to be slightly less. Occasionally blackened material in discrete droplets was seen on either side of the nodes of Ranvier and less frequently in other parts of the sheaths.

In both groups nerves prepared by the Busch technic were infrequently found to show the diffuse type of precipitate. When present, it was slight in amount and consisted mostly of black balls which had no particular distribution. Perineural fat when present was intensely stained. The most consistent finding was the presence of discrete droplets of blackened material which were usually found at the nodes of Ranvier (fig. 4). Nerves containing many such masses of black deposit took on a "speckled" appearance. At times nerves were seen in which no blackening was observed,

---

Fig. 1. Photomicrograph of an unstained gross preparation cleared with cedar oil to show position, type and extent of hemorrhagic process in the brain. Approximately × 8.

Fig. 2. A—Photomicrograph of section of sciatic nerve of rat 3828 (deficient group) showing the diffuse type of precipitate seen in Marchi preparations. × 38.

B—Photomicrograph of section of sciatic nerve of rat 3915 (normal control) showing diffuse precipitate. Marchi × 38.

Fig. 3. A—Higher power of section of sciatic nerve shown in A, figure 2, showing the flaky, and vacuolated masses of black deposit. Marchi × 300.

B—Higher power of section of sciatic nerve shown in B, figure 2. × 300.

Fig. 4. A—Photomicrograph of section of sciatic nerve of rat 3417 (deficient group) showing the nodal type of staining consistently found in Busch preparations. × 75.

B—High power photomicrograph showing nodal droplets of stained material. Rat 3417. × 300.

Fig. 5. Rat 3781. Photomicrograph showing areas of destaining of a normal sciatic nerve. Spielmeyer preparation. × 112.

Fig. 6. Rat 3867 (recurrent group). Photomicrograph of area of hemorrhage in the pons showing phagocytes containing much ingested fatty material. Busch preparation. × 300.

Fig. 7. A—Rat 3660 (deficient group). Photomicrograph of section of brain showing cellular damage in pons. Nissl preparation. × 150.

B—Rat 4172 (recurrent group). Photomicrograph of section of brain showing cellular damage in pons. Nissl preparation. × 300.

Fig. 8. Rat 3653 (recurrent group). Photomicrograph showing area of scarring involving the chief vestibular nucleus and nucleus solitarius. Nissl preparation. × 56.

Fig. 9. Pigeon 205. Photomicrograph of section of brain showing area of hemorrhage of both the perivascular and diffuse type. Nissl preparation. × 38.

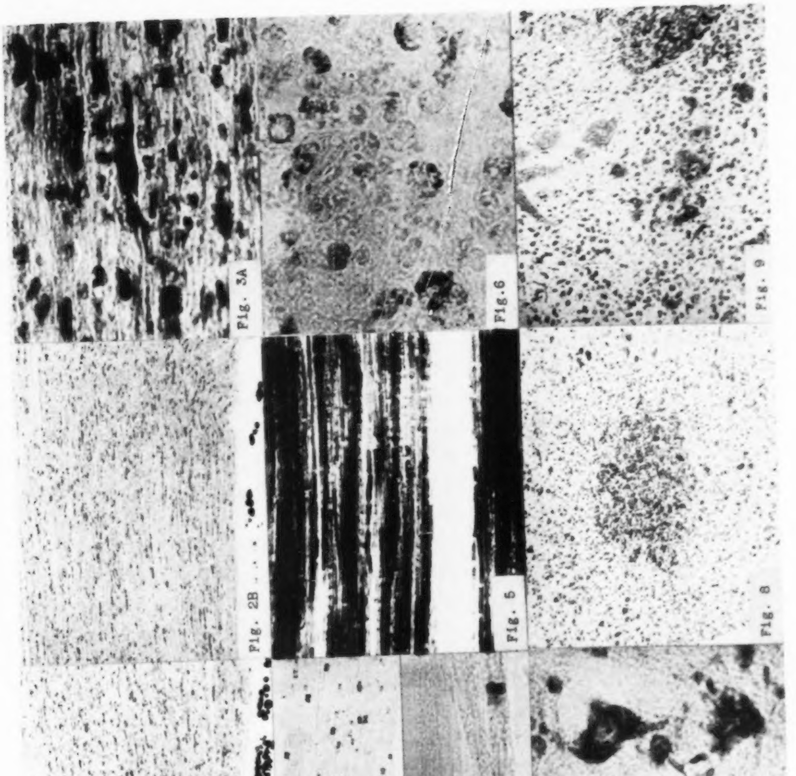


Fig. 1
Fig. 2A
Fig. 2B
Fig. 3A
Fig. 3B
Fig. 4A
Fig. 4B
Fig. 5
Fig. 6
Fig. 7A
Fig. 7B
Fig. 8
Fig. 9

although if perineural fat was present it was intensely stained. In order to check the technic the left sciatic nerves of five normal animals were severed under anesthesia and were injured by pulling and squeezing. After a suitable period of time, the animals were killed and the nerves removed, prepared by the Busch method and the comparison between right and left nerves made. In all severed and injured nerves typical degenerative changes were observed, while in control nerves a variable amount of discrete black droplets was seen at the nodes of Ranvier.

Preparations made by the sudan III method showed no evidence of fatty material within or without the sheaths with the exception of a few fibers showing changes typical of Wallerian degeneration in both the deficient and control groups. The nerves of animals in the recurrent group showed no changes which would differentiate them from other individuals in either group.

Material from both the deficient group and control group A prepared by the Nissl and hematoxylin-eosin methods showed a slight increase in neuroglial elements.

Nerves prepared by the Spielmeyer myelin sheath technic (Romeis, 1928) showed a variation in the resulting picture which could be intensified by variations in the time in the iron alum bath, the time washed after this bath, and the type of dehydration used. Carbol-xylol, full strength (1:3), or half strength (1:6), produced variable amounts of destaining which gave the nerve the appearance of severe myelin change. Figure 5 shows a section of normal sciatic nerve, from a control animal, which has been dehydrated in carbol-xylol. Its similarity to the picture usually described as degeneration is evident. Comparison was made of sections of the same nerves by the sudan III method. In no case was any evidence of myelin change observed. Zimmerman (1932) has pointed out similar changes in the spinal cords of dogs on a diet deficient in the vitamin B complex which he attributes to differences in technic.

Microscopic findings in the central nervous system. Marchi preparations of the brains and spinal cords of animals in both the deficient and control groups showed at all times a variable amount of diffuse blackening which appeared to have no particular distribution. The white matter of the spinal cord in particular, evidenced many black ringlets surrounding the axons. These ringlets appeared to have no particular distribution and were found at various levels of the cord. In the deficient group an occasional cell which was thickly incrusted with blackened material was observed in areas of marked congestion or frank hemorrhage in the pons, medulla, or cerebellum. In the recurrent group, in areas of partially absorbed hemorrhage, innumerable fat-granule cells and swollen oligodendroglia were seen which contained a great deal of fatty material. Perivascular deposits of blackened material were occasionally seen in the pons and medulla.

Sections prepared by the formalin-Marchi method showed the same distribution of blackened material as was observed in Marchi preparations, but the amount appeared to be slightly decreased.

Busch preparations of the brains and spinal cords of animals in both the deficient and control groups showed no diffuse precipitate. Changes were observed in only the recurrent group in areas of partially absorbed hemorrhage in the pons, medulla, or cerebellum, and consisted of blackened fatty material contained in fat-granule cells in this region (fig. 6). Perivascular deposits of blackened material were occasionally seen.

The only changes observed in preparations by the sudan III method were found in the recurrent group in areas of partially absorbed hemorrhage in the pons, medulla, or cerebellum. These consisted of fat-granule cells and swollen oligodendroglia containing a great deal of ingested fatty material.

Brains and spinal cords prepared by the Nissl or hematoxylin-eosin methods showed occasional shrunken, deeply stained cells which had no particular distribution. These cells were observed in both the deficient and control groups. Generalized congestion was observed in the brains and spinal cords of the deficient group and control group A. No other changes were observed which were common to both groups. In the deficient group, however, changes were usually marked and were confined to the pons, medulla, and cerebellum. Disseminated foci of hemorrhage of one or both sides which varied in size and position were observed to affect most consistently the regions of nucleus of Deiters, the nucleus of Bechterew, the chief vestibular nucleus and the nucleus solitarius. In a number of cases, the nuclei dentatus, globosus, emboliformis and tecti of the cerebellum, the restiform body, the dorsal cochlear nucleus, and the nucleus of the abducent nerve were involved and, in two cases, the nucleus of the trapezoid body and the accessory superior and superior olivary nuclei contained hemorrhagic foci. In the majority of cases these foci of hemorrhage appeared acute and no evidence of phagocytosis was observed. However, in the brains of animals in the recurrent group the foci were more widespread and innumerable fat-granule cells and swollen oligodendroglia were observed in and surrounding the area of partially absorbed hemorrhage. In those brains which did not show frank hemorrhage, marked congestion was observed in these areas. In some cases, although the hemorrhage was usually bilaterally symmetrical, congestion was seen on one side and frank hemorrhage on the opposite side. The hemorrhages were of two types: large massive hemorrhage obliterating all tissue elements, and small punctate or perivascular hemorrhages. The former was more often seen than the latter.

Cellular damage was consistently present in the brains of animals in the deficient group which had shown symptoms for some time. The extent of this damage appeared to vary with the length of time the animals were on

experiment after the development of symptoms. Thus, it was least severe or not present at all in animals which became moribund immediately after developing symptoms, and most severe in animals in the recurrent group. The cellular damage bore no evident relation to the hemorrhagic process since it was found in animals whose brains showed only marked congestion as well as in those showing frank hemorrhage. The cellular changes were most consistently seen on one or both sides in the nucleus of Deiters, nucleus of Bechterew, chief vestibular nucleus, and nucleus solitarius. However, it was frequently encountered in the cerebellar nuclei, the restiform body, the dorsal cochlear nucleus, and in two cases in the nucleus of the trapezoid body and the accessory superior and superior olivary nucleus. The changes consisted of shrunken, deeply stained cells which were devoid of processes and which showed at times pericellular encrustations and of large pale-staining vacuolated cells with granulated or clumped Nissl substance and atypically placed nuclei (fig. 7). Occasionally cells with ruptured cell membranes were seen but these were infrequent. Chromatolysis of Purkinje cells was occasionally observed in the vermis and floccus of the cerebellum. That permanent damage could result from the hemorrhagic process was evidenced by one case which showed an area of scar tissue on either side in the area of the chief vestibular nucleus and nucleus solitarius (fig. 8). Neuroglia were increased in these areas but particularly so in the brains of animals in the recurrent group, which showed at times perivascular reaction and, in cases of absorbed hemorrhage, a netlike arrangement of neuroglial fibers which gave a gelatinized appearance.

These findings were further substantiated in the case of a few pigeons which had shown marked symptoms of the spastic form of beriberi. Osmic acid and sudan III preparations gave no indication of myelin damage in the peripheral nerves (sciatics and brachial plexuses), but in the central nervous system disseminated foci of hemorrhage were observed in the pons, medulla, or cerebellum, and to a lesser extent in the optic lobes and cerebral hemispheres. Cellular damage of the same type as found in rat brains in similar areas of the brain was observed in Nissl preparations.

Discussion. In view of the conclusions of numerous investigators that myelin degeneration of the peripheral nerves is a result of vitamin B-deficiency, one hesitates to make a definite statement that such conclusions may be in error. If the amount and distribution of the blackened material in the Marchi preparations of nerves from the deficient group were considered alone, it is probable that a myelin degeneration might be suggested. However, when one is unable to differentiate between these preparations and the Marchi preparations from the three control groups there appears to be no basis for such a diagnosis. It seems more reasonable to question the adequacy of the Marchi method for the demonstration of primary or

periaxillar degeneration. The Busch technic gives preparations which are less likely to be diagnosed as degeneration and these likewise show no significant differences among the various groups. Finally, the failure of sudan III to demonstrate fat droplets within nerves of the deficient group makes it seem extremely doubtful if myelin degeneration of peripheral nerves is essentially a result of vitamin B-deficiency in rats and pigeons.

In sharp contrast with the negative findings in the peripheral nerves are the definite changes found in the central nervous system. These changes assume importance when one remembers that the areas affected are intimately concerned with the equilibration and coördination of the individual. Of the areas most consistently affected, the nucleus of Deiters acts as a center for equilibratory control of the muscles in their relation to the semicircular canals, utricle, and saccule, while the chief vestibular nucleus and the nucleus of Bechterew are concerned with similar functions. The nucleus solitarius is concerned with the transmission of gustatory impulses from the tongue (Tilney and Riley, 1928).

The importance of the changes observed in the pons, medulla, or cerebellum is emphasized by the recent studies on the physiological action of vitamin B. Kinnersley and Peters (1929) have reported an increase in the lactic acid content of the brain in pigeons on a polished rice diet which was a concomitant of the symptoms and disappeared shortly after the administration of vitamin B. Later (Kinnersley and Peters, 1930) these workers found that the increase in lactic acid was localized in the lower parts of the brain (brain stem) and was found only in this area in the period preceding symptoms. Gavrilescu and Peters (1931) have reported a lowered power of oxygen uptake, in vitro, in the brains of pigeons on a polished rice diet which was particularly seen in the lower parts of the brain. Church (1933) has reported a variable degree of nystagmus in rats which reaches its maximum two or three days before the appearance of the usual neuromuscular symptoms and which shows a rapid change toward normal following treatment with brewer's yeast or the heat-labile fraction.

Although injury to the vestibular, cochlear, or cerebellar nuclei could theoretically produce most of the symptoms characteristic of vitamin B-deficiency it is not believed that these symptoms are the result of actual structural change. The rapid disappearance of the symptoms following the administration of vitamin B to deficient animals is incongruous with such a belief just as it is entirely incongruous with the association of the symptoms with myelin degeneration of the peripheral nerves. It is believed, however, that the injuries which are apparent in the central nervous system may be considered as an index to the location of the functional disturbances which produce the symptoms of vitamin B-deficiency.

SUMMARY

1. A study has been made of the peripheral and central nervous systems of a deficient group of rats whose diet was free from vitamin B but contained an adequate amount of vitamin G, and of three control groups whose diets contained adequate amounts of both B and G vitamins.

2. In the peripheral nervous systems the following were observed:

a. Blackened material of the same type, amount and distribution was observed in osmic acid preparations of the sciatic nerves and brachial plexuses of animals in both the deficient and control groups.

b. Nerves stained by the sudan III method at no time showed evidence of degenerative changes in either group.

3. In the central nervous system the following were observed:

a. Disseminated foci of hemorrhage or intense congestion of one or both sides were found to involve the nucleus of Deiters, the chief vestibular nucleus, the nucleus of Bechterew, and the nucleus solitarius in approximately 75 per cent of the animals in the deficient group.

b. Cellular damage, which appeared to vary with the length of time the animal was left on experiment after the development of symptoms, was observed most consistently in the region of the nucleus of Deiters, the chief vestibular nucleus, the nucleus of Bechterew, and the nucleus solitarius.

4. The data indicate that the site of the lesion responsible for the symptoms associated with a lack of vitamin B in the rat is in the central rather than in the peripheral nervous system.

REFERENCES

Church, C. F. 1933. Proc. Soc. Exp. Biol. and Med., **30**, 1158.
Cully, P. G. 1927. Quart. Journ. Exp. Physiol., **17**, 65.
Duncan, D. 1931. Arch. Neurol. and Psychiat., **25**, 327.
Eijkman, C. 1897. Virchow's Arch. f. Path. Anat., **148**, 523.
Findlay, G. M. 1921. Journ. Path. and Bact., **24**, 175.
Gavrilescu, N. and R. A. Peters. 1931. Biochem. Journ., **25**, 1397.
Harris, L. J. 1932. Annual review of biochemistry. Standford U. Press, **1**, 337.
1933. Ibid., **2**, 253.
Kingery, H. M. and J. B. Kingery. 1925. Anat. Rec., **29**, 364.
Kinnersley, H. W. and R. A. Peters. 1929. Biochem. Journ., **23**, 1126.
1930. Ibid., **24**, 711.
Kon, S. K. and J. C. Drummond. 1927. Biochem. Journ., **21**, 613.
Kruse, H. D. and E. V. McCollum. 1932. Journ. Amer. Med. Assn., **98**, 2201.
McCarrison, R. 1921. Studies in deficiency disease. London, H. Frowde, Hodder and Stoughton.
Moore, C. U., J. L. Brodie and R. S. Hope. 1927. This Journal, **82**, 350.
Pappenheimer, A. M. and M. Goettsch. 1931. Journ. Exp. Med., **53**, 111.
Romeis, B. 1928. Taschenbuch der Mikroskopischen Technik, 12 Aufgabe p. 462.
Stern, R. O. and G. M. Findlay. 1929. Journ. Path. and Bact., **32**, 63.
Tilney, F. and H. A. Riley. 1928. The form and functions of the central nervous system. 2nd ed., Hoeber, p. 313.
Vedder, E. B. and E. Clarke. 1927. Phillipine Journ. Sci., **7**, 423.
Voegtlin, C. and G. C. Lake. 1919. This Journal, **47**, 558.
Woollard, H. H. 1927. Journ. Anat., **61**, 283.
Zimmerman, H. M. and E. Burack. 1932. Arch. Path., **113**, 207.

THE OCCURRENCE OF CITRIC ACID IN URINE AND BODY FLUIDS

WALTER M. BOOTHBY AND MILDRED ADAMS

From the Section on Clinical Metabolism, The Mayo Clinic and The Mayo Foundation, Rochester, Minnesota

Received for publication October 9, 1933

Amberg and McClure, in 1917, first demonstrated the presence of citric acid in normal urine of man in quantities ranging around 0.5 gram per liter.[1] Salant and Wise, in the previous year, had shown its presence in the urine of swine; the quantity was increased by the administration of large amounts of citric acid but the substance was still present when no citric acid was administered. In 1921 Amberg and Maver reported the isolation of citric acid from urine of man. Fasold in 1930 again isolated citric acid from the urine and proved it to be such by the melting point and other criteria. The presence of citric acid in milk and milk products had been shown as early as 1888 by Henkel and Soxhlet. However, little attention had been directed to the factors influencing the presence of citric acid in urine of man, on account of the difficulties of the method, until Thunberg in 1929 developed his enzymatic methylene blue method.

Method. Thunberg's method, although tricky, is rapidly carried out and under most conditions is delicate; this is the method which we have used with only the following slight modifications:

Shelled cucumber seeds, 3 grams, were ground in a mortar with 30 cc. triply distilled sterilized water. This mixture was centrifuged, keeping the suspension cold by an ice jacket around the centrifuge cup, and after this the enzyme solution was carefully separated from the fat and the solid residue. The extract of cucumber seeds thus prepared was kept in the ice box over night, before it was used. As a result of this delay before using, the enzyme solution became more stable than if it had been used immediately after its preparation, and also gave a much longer decolorization time for the blanks. This increase in decolorization time of the blank apparently was due to destruction of some of the substances present in the extract of cucumber seed, which caused decolorization of methylene blue even in the absence of citric acid. The citric acid solution was made up fresh each day that the determinations were to be made and was prepared by dissolving 25 mgm. citric acid in 50 cc. triply distilled, sterilized water; the dilu-

[1] All quantities of citric acid are based on the crystalline form containing 1 molecule of water. Molecular weight = 210.

tions from this were made with a phosphate buffer solution prepared by mixing equal volumes of 0.125 molar K_2HPO_4 and KH_2PO_4 giving a pH of about 6.7. Using this more acid phosphate solution and the enzyme prepared as has been described we have been able to obtain more constant citric acid curves, and because of the removal of interfering substances which react with methylene blue it is possible to use more dilute solutions of methylene blue. For most purposes methylene blue solutions 1:50,000 were used but when the concentration of citric acid was very low, as in the urine of some of the dogs, methylene blue solutions 1:70,000 were used to make possible the detection of smaller amounts of citric acid. The urines to be investigated were also diluted with the same phosphate buffer mixture.

Reported determinations on various body fluids. Thunberg and his pupils, especially Östberg, have made extensive studies on the citric acid in urine and body fluids. The reader is referred to the exhaustive paper by Östberg for a detailed report of the literature. Citric acid in concentrations varying between 3 and 10 mgm. per cent have been found in the aqueous humor of the eye (Grönvall), spermatic fluid (Scherstén), amniotic fluid (Nitzescu and Georgescú), cerebrospinal fluid (Benni) and human milk (Jerlov). The study of the citric acid content of the blood presented greater difficulties; the latest work by Thunberg shows that it is present in concentration of between 1 and 4 mgm. per cent in blood serum.

The quantity of citric acid excreted in the urine is increased by a vegetable diet (Fasold) and by the administration of sodium bicarbonate (Östberg). It is decreased by the administration of NH_4Cl or $CaCl_2$ and frequently decreased in diabetic coma (Östberg).

This type of evidence suggested to Östberg that the chief function of citric acid in the metabolism of man is to play a part in the maintenance of the acid base equilibrium of the body by conserving the elimination of inorganic acid radicals following administration of excess base, in a manner similar to the conservation of base by the formation of ammonia in acidosis. It is obvious, of course, that citric acid can play such a part efficiently in the presence of excess base, because it is a tribasic acid, with all three of its carboxyl groups almost completely neutralized at a pH at or above 7.0, so that 1 gram under such conditions will be able to neutralize approximately 140 cc. of N/10 alkali.

Present investigation. Although we in no way deny that under certain circumstances citric acid may play a part in acid-base equilibrium, the data which we will present show that this is not invariably the case. On the other hand, the fact that there is such extensive distribution of citric acid in the various body fluids, such as the cerebrospinal fluid, the amniotic fluid, the aqueous humor of the eye, the blood, the milk, and the urine, suggests that citric acid is an intermediate product of normal metabolism, at least in some of the organs of the body and as such probably has an im-

portant part. Our investigations have been directed toward discovering what this purpose is; our results, unfortunately, have been indefinite, except to show that the amount of citric acid in the urine of the dog gradually increased during a prolonged fast of three weeks, thus conclusively proving that its origin is not necessarily from ingested carbohydrate, or from the citric acid content of the food, and, therefore, that it is, in fact, an intermediate product of metabolism.

Experiment 1. The following experiment was carried out on a healthy male laboratory technician while he was eating the various foods served in his home. His age was thirty-four years; his weight, about 216 pounds (98.2 kgm.), and his height, 6 feet, 2 inches (188 cm.). During a preliminary period of seven days he excreted about 0.7 gram of citric acid daily. Throughout the next nine days he took $NaHCO_3$, 5 grams twice daily; the quantity of citric acid definitely increased and averaged about 1.2 grams daily, but the quantity was quite irregular and on one day was as high as 1.9 grams. The pH increased from about 5.5 to around 7.3, and the quantity of ammonia decreased from about 0.55 gram to less than 0.20 gram. The following day two doses, 3 grams each of NH_4Cl were given, and the next day one dose of 5 grams and one of 3 grams. This caused rapid decrease in elimination of citric acid within a few hours, from 0.10 to 0.03 per cent, and subsequent to the last dose administered the concentration, for a few hours, decreased to 0.019 per cent. The lowest quantity eliminated in twenty-four hours was on the third day, the day after administration of NH_4Cl had been stopped, when the quantity decreased to 0.35 gram, the pH dropped to 5.0, and the quantity of NH_3 increased to more than 1.0 gram. After an interval of eighteen days, NH_4Cl was again given for one day, followed by $NaHCO_3$ during the next nine days, with one day's intermission, with essentially the same results as just noted. These observations are in accord with those found by Östberg; our high and low values are not as extreme as some he obtained; on the other hand, the amounts of $NaHCO_3$ and of NH_4Cl administered by us were less.

However, during the intervening period of eighteen days, when his diet was normal and he was undisturbed by the administration of acids or alkalies, the quantity of citric acid in the urine fluctuated between 0.6 and 0.9 gram; at the same time there was an interesting and suggestive correspondence in that these fluctuations followed closely the normal fluctuations in the excretion of urea, and less closely the excretion of uric acid. On the other hand, no indication of any relationship was obtained in this experiment with the excretion of any of the following substances: total organic acids, lactic acid, total and free benzoic acid, hippuric and glycuronic acids, total and preformed creatinine, the inorganic phosphates, and the total sulphates.

Experiment 2. In order to study the effect of a long-continued submaintenance diet, we observed an obese woman, aged twenty-five years, who weighed, at the beginning of the experiment 192 pounds (87 kgm.) and whose height was 5 feet, 2 inches (158 cm.). She was given the following diet: 70 grams protein, 16 grams fat, 40 grams carbohydrate. This diet totalled 600 calories, and was planned to cause rapid reduction in weight without much loss of protein. Although the diet did not contain much fat, the effect was definitely ketogenic because its low calorie value resulted in combustion of considerable amounts of body fat, as evidenced by the rapid loss in weight, the increase in the excretion of ammonia and organic acids which were determined daily, and the increase in acetone bodies. Study of the curves disclosed certain facts: First, as the curves for ammonia and organic acids rose to more

than 1.7 grams and 1100 cc. N/10 respectively, there was a fairly corresponding fall in the citric acid curve; during the first two weeks of the experiment the ketosis may have been a factor in the decrease in elimination of citric acid. The value for citric acid did not, however, decrease below 0.1 gram, and then again it rose in spite of the ketosis. Second, in addition to this effect there was an occasional parallelism between the curve representing citric acid and that representing urea. This parallelism suggests that in addition to the effect of acid and base on the elimination of citric acid there may be another influence which produces less marked fluctuation, and which in some way is exerted by protein metabolism.

Experiment 3. A patient with lymphoblastoma, probably Hodgkin's disease, was studied in conjunction with Doctor Desjardins to see if the effect of heavy roentgen therapy would produce metabolic changes that would affect elimination of citric acid. Following the beginning of treatment there was a definite increase in elimination of organic acid accompanied by increase in elimination of ammonia. The pH of the urine remained constant around 6.5, except on two days, due to decomposition of urea into ammonia from standing. The citric acid was at first apparently unaffected by the increased formation of organic acids; however, the total amount of citric acid excreted before treatment was rather low, 0.1 gram. A part of the high excretion of organic acid was due probably to relative starvation, for the roentgen therapy caused considerable reaction, with complete loss of appetite, and nausea. Another part may have been due to increased formation of unknown organic acids as a result of the breakdown of tissue from the roentgen therapy. After eight days, the patient improved somewhat and was able to eat; this caused decrease in the ketosis and elimination of citric acid markedly increased. Again, in this experiment, sudden marked increase in elimination of urea was accompanied by coincident increase in elimination of citric acid. There were also more or less parallel fluctuations with the elimination of citric acid in the elimination of preformed creatinine, total amino acid, and uric acid. Part of these fluctuations may have been due to incomplete collection of urine, for the patient was very ill. Since the excretion of citric acid followed these large variations in elimination of urea it is possible that the coincident variation in elimination of citric acid may have been here, too, more than accidental.

Experiment 4. Elimination of citric acid was one of the many factors followed in an exhaustive experiment in balanced metabolism which lasted about three months. The subject was a female dog, weighing 36 pounds (16 kgm.). The details of this experiment will be published elsewhere. Throughout the experiment the dog excreted only small quantities of citric acid; the quantity fluctuated irregularly around 0.06 gram daily until the dog was taken ill with severe pyometritis. As a result of the infection, with formation of abscess, the temperature rose to 105.4°F. and for three days the animal refused all food. During this severe, and unexpected illness, excretion of citric acid increased 300 per cent, and on the third day during which the animal completely refused food, reached 0.18 gram. At the same time the total respiratory quotient, obtained for the entire twenty-four hours, decreased to 0.72, and the non-protein respiratory quotient to 0.69, which indicated that no carbohydrate (except that arising from protein and the glycerol fraction of the fat), was entering into the metabolic processes at the time the elimination of citric acid was highest. During this period of starvation the quantity of citric acid increased from 10 to 25 cc. N/10, while at the same time excretion of organic acid increased from around 200 cc. N/10 to more than 280 cc. With improvement, and resumption of eating, both values decreased. In this instance, therefore, there was not an inverse relationship between elimination of citric acid and of other organic acids. The

important new fact accidentally discovered in this experiment is that increase in elimination of citric acid may be marked in complete starvation and at a time when the non-protein, twenty-four hour respiratory quotient, confirms the fact that carbohydrate, as such, is not being burned. This indicates that citric acid eliminated in the urine not only does not come from ingested food, but also that it is not necessarily an end-product of carbohydrate metabolism.

Experiment 5. When thyroxin, in doses of either 10 mgm. or 20 mgm., was given intravenously to a dog on a normal diet, there was no increase in elimination of citric acid. There was probably a slight decrease, but this could not be determined with certainty because even before thyroxin had been given, the amount of citric acid excreted was at about the lowest concentration that could be detected. The increase in volume of urine that followed administration of thyroxin may only have decreased the concentration to a point too low to be determined. Following both injections of thyroxin the characteristic increase in production of heat developed, as well as the characteristic temporary increase in the elimination of total nitrogen, creatinine and creatine; there was also a slight increase in the total excretion of benzoic acid. It can be concluded, however, that neither the thyroxin directly, nor the type of increased protein metabolism, temporarily caused by the administration of thyroxin, caused increase in elimination of citric acid. It cannot, however, be necessarily concluded that there was not increased formation of citric acid as an intermediate step in metabolism, because thyroxin might also have increased its rate of destruction.

After the effect of the second dose of thyroxin had passed off the dog was fasted. For three days the quantity of citric acid in the urine remained at the same low level, around 0.03 gram daily. On the fourth day, which corresponds to the time at which available stores of carbohydrate usually are depleted, there was marked increase in elimination of citric acid to 0.11 gram; elimination reached an average of 0.24 gram daily for the fifth and sixth days of the fast. Thyroxin, 15 mgm., was then given intravenously, the fast being continued, and there was a sharp drop in elimination of citric acid to the low level that had obtained before the fast. This was followed by gradual increase in the next three days to the high, fasting level. On the first day of the partaking of food (standard diet) excretion of citric acid again decreased to the level that has obtained before the fast, as might have been expected. The unexpected feature was that during the next four days, excretion of citric acid again increased to around 0.18 gram, and on the fifth day the quantity suddenly decreased to below the detectable level and remained there for nine days, after which citric acid again was excreted at the low level of 0.03 gram daily, which had obtained before the fast.

About four weeks after the ending of the first fast the dog was fasted again. This time there was a slower and less marked increase in the elimination of citric acid, which, after twelve days, amounted to 0.12 gram daily. Fifteen milligrams of thyroxine were again given intravenously followed by a slight decrease in citric acid in the next three days to 0.09 gram, and on the fourth day suddenly increased to the unusually high level for a dog of 0.62 gram; the next day there was a slight decrease to 0.44 gram. As the dog was becoming somewhat weak as a result of the eighteen-day fast, food was started. This time instead of giving the standard diet the food was practically limited to carbohydrate in the form of cracker meal which was moistened with a little milk. On the first day of food the citric acid decreased to 0.10 gram for one day and on the second showed a tremendous increase to 0.92 gram and on the following day decreased to 0.23 gram and then slowly decreased to below the detectable limits, only to return again to the original low prefasting level.

Comment on experiments 4 and 5. During the periods of fasting of the dogs in both experiments, the elimination of citric acid in the urine increased. The twenty-four hour nonprotein respiratory quotient of 0.69 determined in experiment 4 confirmed the fact that, although in that experiment the fast was of short duration, carbohydrate as such was not being burned. The two fasts in experiment 5 were sufficiently long to have allowed to be used up all carbohydrate that could have been considered as a true reserve. During the entire second fast of nineteen days the dog excreted a total of 2.26 grams of citric acid. No evidence is available which would suggest the possibility that this amount could come from citric acid previously stored in the body tissues. The conclusion seems sound that the citric acid eliminated in the urine does not necessarily arise from ingested carbohydrates and can come, at least in part, from the metabolic breakdown of either fat or protein.

In this connection some recent experiments of Amberg are interesting. While working in this laboratory with the pentabromacetone method Amberg found a definite increase in citric acid output from the administration of $NaHCO_3$ which, however, was not further increased by the administration of 200 grams glucose in addition to his regular diet as illustrated by the following experiment: On the control day he excreted 0.24 gram citric acid; first day on $NaHCO_3$ he excreted 0.75 gram; second day on $NaHCO_3$ he excreted 0.86 gram; third day on $NaHCO_3$ plus 200 grams glucose he excreted 0.75 gram. Similar results were obtained on repetition of such experiments on other volunteers. These experiments are further evidence that the elimination of citric acid is not materially affected by ingested carbohydrate.

Experiment 6. We have examined the urine of a dog for the presence of citric acid, following removal of the liver in four instances. In all cases, practically no citric acid was found in the urine for the first four hours after hepatectomy; following this there was a very definite increase, usually to a level much higher than the normal amount for a dog; for example, one dog eliminated as much as 44 mgm. per 100 cc. and 118 mgm. per hour; the total for a period of thirteen hours amounted to 1.13 grams. The fact that following removal of the liver there is, after a brief interval, an increase in the amount of citric acid eliminated, suggests that possibly citric acid in the dog is normally utilized or destroyed by the liver. Immediately following suprarenalectomy, on the other hand, elimination of citric acid by the dog usually varied only slightly, to the degree encountered under normal conditions.

Experiment 7. McClure and Sauer showed citric acid to be present in all of thirty-nine children of various ages from seven months to fourteen years. We have studied the urine of seven newly born infants, before they received food, and have found citric acid present in all. The concentration was between 13 and 40 mgm. per cent, which is a considerably larger concentration than that found by Östberg in four cases. We have also confirmed the fact that amniotic fluid contains citric acid in a concentration around 7 mgm. per cent.

Experiment 8. We have made sixty-three determinations on cerebrospinal fluid.

The mean concentration in all determinations was 5.0 mgm. per cent. The distribution was that shown in table 1, from which it is obvious that the series is still too small to allow of an attempt at interpretation of the cases at the outer ends of the normal distribution.

Experiment 9. Östberg examined the urine of 153 patients admitted to the wards of University Hospital, Lund, Sweden, for the presence of citric acid. We have made a similar study on patients at The Mayo Clinic (table 2). The distribution of the two series is strikingly similar.

In Östberg's series there were several patients with diabetes, two of whom entered the hospital with a very high degree of acidosis; the latter, at first, did not excrete detectable amounts of citric acid. As the condition of the patient was brought under control by treatment, and the acidosis decreased, citric acid appeared in the urine. We have observed one similar case; however, in another case we found citric acid present even while the patient was in coma. As has been noted, we have found that the degree of acidosis produced by a ketogenic diet did not necessarily cause disappearance of citric acid although the amount excreted may sometimes be a little decreased. One gram of citric acid may be excreted during the day when the pH of the urine is as low as 5.2, and when the subject is on a ketogenic diet the fluctuations in excretion of citric acid frequently show little if any correlation with the pH.

TABLE 1

Citric acid in cerebrospinal fluid

MGM. PER CENT	DETERMINATION
0 to 1.9	1
2.0 to 3.9	17
4.0 to 5.9	35
6.0 to 7.9	4
8.0 to 9.9	3
10.0 to 11.9	3
Total............	63

Mean = 5.0 mgm. per cent citric acid.

TABLE 2

Citric acid in urine of patients in hospital

ÖSTBERG'S SERIES, PATIENTS	OUR SERIES, PATIENTS	GRAM IN 24 HOURS
32	35	None or below that which could be detected
29	30	Trace to 0.09
58	37	0.10 to 0.19
14	20	0.20 to 0.29
12	17	0.30 to 0.39
3	6	0.40 to 0.49
3	3	0.50 to 0.59
0	3	0.60 to 0.69
0	1	0.70 to 0.79
1	0	0.80 to 0.99
1	1	More than 1.00

In our group of cases, summarized in table 2, there were many different diseases, and also a fairly representative group of patients who had various types and degrees of renal disease, of hypertension, of exophthalmic goiter, and of cardiac disease. We have carefully reviewed the histories of the

cases, and have been unable to discover any correlation that would be of clinical significance, nor have we as yet encountered any pathologic conditions, with the possible exception of severe diabetic acidosis, which would help in determining the factors which produce the variations in the daily amount excreted. Space does not permit presentation of these data in detail.

Experiment 10. In conjunction with the Section on Urology of The Mayo Clinic we have examined the urine of several patients who had renal calculi, in view of the possibility, as considered by Östberg, that the presence of citric acid in the urine will tend to decrease the development of renal calculi. His suggestion is based on the rather well known fact that the presence of citric acid tends to prevent complete precipitation of calcium salts. In only two cases of renal calculus which were studied was citric acid absent; in some the concentration was rather high at the time of examination. It does not necessarily follow, however, that citric acid was present in the urine at the time the stones were being formed. Because administration of citric acid does not, as a rule, readily increase the concentration in the urine, the only method at our disposal for increasing the concentration of citric acid in the urine is administration of $NaHCO_3$. Since there is a definite impression among urologists that alkaline urine is favorable to formation of stone, one must be cautious in using $NaHCO_3$ as a means of increasing elimination of citric acid. It might readily be that in the formation of this impression no particular attention has been paid by urologists to the possible difference between a urine rendered alkaline by bacterial decomposition of urea into ammonia and production of an alkaline urine by $NaHCO_3$. However, it is seldom possible to obtain a concentration of citric acid of more than 0.1 to 0.2 per cent by means of feeding $NaHCO_3$ and although it is not possible to say just what would be the effect of citric acid on the solubility of calcium salts in the complex system which exists in urine, the concentration of citric acid necessary to produce marked changes in the solubility of calcium salts is generally much higher than this.

SUMMARY

Citric acid usually is excreted in the urine of normal people, under ordinary dietary conditions. The amount so excreted varies around 0.5 gram daily. The concentration is likely to be around 30 mgm. per cent. It is found in the urine of newly born infants. The citric acid content of the urine of more than 300 patients has been studied, including the studies made by Östberg, and we have been unable to read any practical clinical significance into the variations in the daily amount of citric acid excreted.

Although in a few cases the administration of large amounts of citric acid will increase the amount excreted in the urine, more frequently it does not. Administration of physiologically strong base, such as $NaHCO_3$ usually will increase the output from around 0.5 gram to about 2 grams daily. Administration of a strong acid, such as HCl ($CaCl_2$ or NH_4Cl) decreases or stops the elimination of citric acid; moderate ketosis may or may not do so. Citric acid is present only in small amounts, or not at all, in the severe acidosis of diabetic coma. We do not believe that the

evidence warrants the conclusion that the chief metabolic function of citric acid is to maintain acid-base balance.

Citric acid must play some important, although as yet an unknown part in the intermediate steps of metabolism, due to its wide distribution in the various body fluids: blood, spinal fluid, amniotic fluid, spermatic fluid, aqueous humor of the eye, milk and urine. It does not necessarily arise from ingested citric acid or carbohydrate food, for in the dog the amount is increased throughout a prolonged fast. It is also excreted in increased amounts after removal of the livers of dogs, but the amount is not significantly altered as a rule by removal of the suprarenal glands.

REFERENCES

Amberg, S. Personal communication to the authors.
Amberg, S. and M. E. Maver. 1921. Journ. Biol. Chem., **46**, xv.
Amberg, S. and W. B. McClure. 1917. This Journal, **44**, 453.
Benni, B. 1930. Biochem. Zeitschr., **221**, 270.
Fasold, H. 1930. Zeitschr. f. Biol., **90**, 192.
Grönvall, H. 1930. Biochem. Zeitschr., **220**, 82.
Henkel, T. Quoted by Östberg.
Jerlov, E. 1929. Svenska Läk.-Tidning., **26**, 785, 811.
McClure, W. B. and L. W. Sauer. 1922. This Journal, **62**, 140.
Nitzescu, I. I. and I. O. Georgescú. 1930. Compt. rend. Acad. d. sci., **190**, 1325.
Nordbö, R. and B. Schersтén. 1931. Skand. Arch. f. Physiol., **63**, 124.
Östberg, O. 1931. Skand. Arch. f. Physiol., **62**, 81.
Salant, W. and L. E. Wise. 1916. Journ. Biol. Chem., **28**, 27.
Scherstén, B. 1929. Skand. Arch. f. Physiol., **58**, 90.
Soxhlet. Quoted by Östberg.
Thunberg, T. 1929. Biochem. Zeitschr., **206**, 109.
1933. Kungl. Fysiografiska Sallskapets i Lunde Forhandlingar. Nr. 17, **3**.

THE EFFECT OF EXTRACTS OF THE ADRENAL CORTEX ON GROWTH AND THE REPRODUCTIVE SYSTEM OF NORMAL RATS, WITH PARTICULAR REFERENCE TO INTERSEXUALITY

EVELYN HOWARD AND ARTHUR GROLLMAN[1]

From the Laboratories of Physiology and Pharmacology and Experimental Therapeutics, The Johns Hopkins University, School of Medicine, Baltimore, Md.

Received for publication September 27, 1933

A relationship between the adrenal cortex and the reproductive system has long been suspected because of the following observations: 1, a sexual dimorphism in adrenal structure; 2, sex differences in the size of the adrenal cortex; 3, adrenal changes following castration; 4, failure of the reproductive system in adrenalectomized animals; 5, clinical observations of functional and anatomical modifications of the reproductive tract concomitant with hypertrophy or malignancy of the adrenal cortex. These various lines of evidence are suggestive of a gonadal-adrenal inter-relationship, but, alternatively, in each case the effects observed are explicable as non-specific concomitants of widespread bodily changes. Consequently it is desirable that more direct tests of a gonadal-adrenal inter-relationship be obtained. This has been made possible by the preparation of a relatively pure extract of the adrenal cortex (Grollman and Firor, 1933b) which is active in maintaining life and normal reproductive activity in adrenalectomized mammals.

In the present study we have tested the effects of this cortical extract on the reproductive system of normal young rats, and have obtained observations on ovarian activity as evidenced by the oestrous cycle, and by pregnancy, and on normal growth of the gonads as well as their compensatory hypertrophy. Observations on general body growth are included. The results show that there is no appreciable effect of a moderate excess of the cortical hormone on the reproductive system or on body growth.

It has been suggested that the adrenal cortex contains two hormones, one essential to life and another reacting on the reproductive system. This idea is not supported by our experiments. It might be argued that the extracts which we have used, being more highly purified than those used by previous workers, have been deprived of some sex stimulating principle

[1] Aided by a grant from the Committee on Scientific Research of the American Medical Association.

in the process of purification. This argument, however, is contradicted by the fact that the extracts which we have used maintain adrenalectomized animals in perfect condition insofar as their reproductive system is concerned. Adrenalectomized rats receiving extract have given birth to and reared normal litters while under treatment. Hence, assuming that the adrenals normally regulate the sexual functions, such a regulatory hormone is present in our extracts. On the basis of our present knowledge, there is no evidence for the existence of more than one hormone in the adrenal cortex and this hormone is essential for the well-being of the reproductive system as it is for the other organs and tissues of the body.

Presentation of experiments. Rats were injected intraperitoneally with extract of adrenal cortex prepared according to the method previously described (Grollman and Firor, 1933b). Treated animals were paired with controls of similar age, size, sex, and color, litter mates in most cases. Controls were kept in the same cage with the animals receiving extract. In some cases controls received equal quantities of isotonic saline intraperitoneally; in other cases this was omitted without appreciable change in the results.

In the tables of experimental data, the amount of extract administered is expressed in terms of the original weight of cortex from which it was derived, and is tabulated as administered in grams per day. When administered to 30 to 50 gram rats, doubly adrenalectomized at a single sitting, an amount of extract which corresponds to about 2 to 4 grams of cortical tissue is required for the maintenance of normal growth. This amount of extract may, therefore, be considered as roughly corresponding to that normally elaborated by the unoperated rat. Hence in administering the extract derived from 10 to 34 grams of tissue daily, as used in our experiments, about two to seventeen times the normal physiological requirement of the organism was injected. The cortical extract was made in two strengths, equivalent respectively to 10 and 40 grams of fresh cortex per cubic centimeter. When large doses were used, especially in young rats, the extract was administered in two equally divided doses per day.

The results of the injection of cortical extract on growth and testicular size in normal male rats are shown in table 1, part A. The animals are divided into 4 groups, receiving extract in the amount indicated in the table. In group I the testicular size in the injected animals is slightly greater than the controls, but that this is probably due to natural variation rather than to the treatment is shown by groups II and III which were composed of rats of similar size treated for similar periods of time. Thus during a period in which the young male rat doubles its size, there is no appreciable effect of these doses of the extract of adrenal cortex on body growth or testicular size.

In group IV the animals were treated for a period of 1 month with a

larger dosage of cortical hormone, with similarly negative results. It will be noticed that the average of testicular size of all groups shows a difference of less than 2 per cent from the controls.

These negative findings are in contradiction to results of Müller (1931) but in view of the crudity of his extract and the small amount of data he presents it is probable that his conclusions were based on results from an

TABLE 1

The effect of extract of adrenal cortex on growth of body and gonads in young rats

GROUP NUMBER	CONTROLS				INJECTED				
	Initial age	Initial body weight	Final body weight	Gonadal per cent of final body weight	Dosage of cortex daily	Duration of injections	Initial body weight	Final body weight	Gonadal per cent of final body weight
	A. Males. Averages of 5 to 7 animals per group								
	days	*grams*	*grams*		*grams*	*days*	*grams*	*grams*	
I	10 to 35	36	59	0.753	2 to 10	7 to 18	38	61	0.909
II	21	28.1	53.7	0.876	12.5	7 to 13	28.9	57.3	0.850
III	37	74.2	94.7	1.64	10	8	74.5	91.7	1.44
IV	22 to 28	63.0	164	1.26	20	30 to 32	63.9	178.6	1.24
Average........		51.1	88.3	1.13			52.0	92.1	1.11
	B. Females. Averages of 3 animals per group								
V	21 to 26	33	57.2	0.0236	10	4 to 16	37	60	0.0257
VI	22 to 30	66.6	109.3	0.0353	10	15 to 17	66.7	101.7	0.0331
VII	22 to 30	54.5	161.6	0.0400	10	45	60.2	147.8	0.0459
Average........				0.0330					0.0349
	C. Females. Left ovary only. Averages of 3 animals per group								
VIII	33 to 43	—	154	0.0194	10 to 20	48 to 56	—	149	0.0170
IX	23	—	136	0.0201	10 to 20	48	—	114	0.0180
X	20	—	127	0.0203	10 to 20	57	—	133	0.0154
Average........				0.0199					0.0168

inadequate number of animals, or else were due to non-specific impurities in his extract. It is probable that the findings of Corey and Britton (1931) may be attributed to similar factors. Kaplan (1932) has also been unable to confirm Müller's and Corey and Britton's reports using a lipoid-free extract. These authors did not take into account the presence of lecithin or other lipoids which as Jaffe and Ranssweiler (1926) have shown may induce hypertrophic or atrophic changes in the sex organs after long injection.

The effect of the cortical extract on female rats was also studied. The effects of cortical extract on the oestrous cycles of young rats are shown in table 2. Dosages of hormone were at first the equivalent of 10 grams daily of cortex, and were later increased to 20 grams daily. This having proved

TABLE 2

The effect of extract of adrenal cortex on the oestrous cycle of young rats

AGE INJECTIONS BEGUN	AGE AT FIRST OESTRUS	AGE VAGINA OPENED	LENGTHS OF INDIVIDUAL OESTROUS CYCLES
I. Injected animals			
days	*days*	*days*	*days*
33 ±2	61	61	7-6-6-4-6:5-4
43 ±2	62	44	4-3-5-14-3:7
43 ±2	46	45	3-3-6-5-4-4-5-6
43 ±2	47	46	6-8-6-6-6-4-6:3-6
23	51	51	6-7-7:5-4
23	51	51	6-4-4-6:6-4
23	49	49	6-5-5-5:5-4
20	48	40	3-6-6-6-8:
20	48	39	5-3-4-4-3-4-5:
20	51	47	7-3-4-6-5-5:
10	44	44	7:12
10	53	48	7:6
II. Controls			
	49	47	12-7-6-7-4-6:6
	44	44	4-6-4-4-6-5-5
	82	79	:12-5
	49	48	6-8-6-6-5-5-6:5-4
	44	43	3-2-3-3-2-3-3:18
	45	44	4-2-6-4-4-5:6-4
	51	50	4-4-7-6-5:6-4
	48	40	2-6-2-13:
	46	41	7-6-4-6-5:
	45	44	12:8
Averages:			
Injected........	51	47	5.45
Controls........	50	48	5.58

ineffective in suppressing the oestrous cycle an attempt was made to detect such an effect by reducing the available supply of ovarian hormone by unilateral ovariectomy and, in rats 9, 14, and 16, the dosage of cortical hormone was simultaneously increased to 34 grams daily. However, no appreciable effect on the ovarian activity as manifested in the oestrous cycle could be detected. In table 2 the time of ovariectomy is indicated

by a colon, which separates those cycles completed before the operation, from those cycles completed subsequently.

The oestrous cycle was followed by making vaginal smears twice daily at twelve hour intervals. In taking the smear small glass rods of smooth surface were used, and care was taken to avoid irritation or stimulation of the vagina or cervix which may in itself affect the cycle. The controls were not injected with saline. As shown in table 2, the average cycle lengths were 5.5 days for the animals receiving extract, against 5.6 days for the controls, a difference which is probably insignificant and certainly indicates no suppression of the oestrous cycle. No effect was observed on the age of vaginal opening, or the age of first oestrus. No differences in the type of smear were observed between the control and experimental group.

At the end of this period of observation, animals 23 and 25 (unilaterally ovariectomized) were placed with a male, became pregnant at their first and second oestrous periods thereafter, respectively, and delivered normal litters[2] while receiving extract equivalent to 40 grams of cortex daily. Hence a very considerable excess of cortical hormone did not repress reproductive activity in the normal female rat.

Claus (1931) has reported the production of early sexual maturity and luteinization of immature ovaries after injections of cortical extracts. However, she showed that this reaction is not specific but that the same physiological effects may be obtained after injection of extracts from a variety of tissues, namely, thyroid, epididymis and fish sperm. This reaction was found by Claus to be equivalent to that produced by anterior pituitary and the urine of pregnant women. Hence it is probable that the reaction is caused by a substance of nonspecific nature, and not necessarily a hormone. Later authors (Corey and Britton, 1931, and Migliavacca, 1933) also noted the luteinizing effects of adrenal cortical extracts observed by Claus, but failed to recognize the nonspecificity of this reaction.

The present finding that purified active extracts of the adrenal cortex have no effect on sexual maturity in the rat clearly differentiates the cortical hormone from the widely distributed material responsible for this reaction. The lack of any pubertal stimulation by cortical extracts has also been reported by Gaunt and Parkins[3] (1932), Cleghorn (1932b), and Con-

[2] This is contrary to Britton and Kline's (1933) abortifacient effects which these authors consider a physiological action of the adrenal cortical hormone. We have never observed abortion in a series of over a dozen pregnant animals treated daily with 2 cc. of extract (20 grams of cortex) throughout pregnancy. Britton and Kline's results may be attributed to gross mechanical injury due to the large amounts of extracts which they injected (10 cc. or more) or to the presence of acetylcholine or other noxious impurities present in their extracts. Choline, histamine, and crude tissue extracts in general are known (Backmann, 1921) to induce pronounced uterine contractions.

[3] Gaunt and Parkins (1932) speak of their extracts containing 20 dog units per cubic

nor (1931). Inhibitory effects reported by Connor are not in accord with our findings and are probably due to toxic substances in his extract. The presence of toxic phenolic substances in certain cortical extracts has been demonstrated by Grollman and Firor (1933b); the presence of choline in extracts prepared by the method of Swingle and Pfiffner has been shown by Eagle (1933) while histamine was found in these extracts by Cleghorn (1932a). The presence of these pharmacologically active agents makes it impossible to accept the physiological effects observed following the use of the extracts as specific for the cortical hormone.

Table 1, parts B and C, shows the effect of treatment with cortical extract on the ovarian weights, and it will be seen that there is no consistent difference between the control and experimentally treated animals. The animals listed in part B were killed during the same phase of the oestrous cycle in most cases. Hence it is evident that the normal growth of the young ovaries was not affected by the cortical hormone. Group V was composed of prepubertal females in which the vagina had remained unopened at the termination of the experiment.

It seemed possible that if the adrenal cortical hormone tended to limit the size of the ovary, one might detect an effect on the compensatory hypertrophy of adult ovarian tissue. Accordingly the left ovaries were removed from six rats of 71 to 99 days of age. These rats had previously been subjected to treatment with extract for periods of 48 to 56 days. After intervals of about two weeks, the animals were killed at the same phase of their oestrous cycles, and the right ovaries weighed, after treatment with extract equivalent to 20 to 34 grams of cortex daily. The average ovarian percentage of body weight for six control rats was: left ovaries 0.0198 gram, right ovaries 0.0248. For the treated animals the left ovaries averaged 0.0176 gram, the right 0.0243. Hence no significant effect on the growth of ovarian tissue was obtained.

Sections of pituitaries, thyroids, adrenals, ovaries, and testes revealed no histological abnormalities in the rats treated with extracts of the adrenal cortex.

The weights of the rats at the beginning and end of treatment are included in the tables. Although an adequate supply of cortical hormone is necessary for growth, as shown by numerous assays, it is seen that an excess of cortical hormone has no marked or consistent effect on growth. The slight differences recorded are probably due to natural variation among the animals.

---

centimeter. The method of assay upon which this unitage is based is, however, open to question. Our own assays of preparations of the Swingle and Pfiffner preparations as made by ourselves, in commercial preparations, or as prepared in other laboratories, has failed to reveal the presence of more than one rat unit per cubic centimeter. In our opinion, therefore, the high dosages assumed by Gaunt and Parkins are illusory.

Discussion. Although recent authors on the adrenal have attempted to link the function of the cortical hormone with some specific bodily activity, the attempts have, like their predecessors, been discarded as inadequate. In our opinion the active principle of the adrenal cortex is of the nature of a general tissue hormone. While adrenalectomized animals fail to become pregnant, or, if pregnant before adrenalectomy, fail to lactate or may die at parturition (Firor and Grollman, 1933a) and while pathological changes in the testes and interference with the normal oestrous cycle have been demonstrated by many observers, (including Freed Brownfield and Evans, 1931; Martin, 1932; and Atwell, 1932) nevertheless these changes are only a part of the picture of generally altered body functions. Hence reproductive failure in adrenal insufficiency cannot be taken as evidence of any specific relationship between the gonad and the adrenal cortex.

Histologically there is in some species a definite effect of the gonads on the adrenal cortex. As previously described (Howard, 1927; Deanesley, 1928) there is a temporary sexual dimorphism in the adrenals of mice, due to the presence of the X zone in the young adult female. This difference disappears after prepubertal castration of the male. Pregnancy under some circumstances affects the histological picture in the adrenals in mice, but this is not a necessary concomitant of pregnancy. It has been shown in the work of Donaldson (1919), Hatai (1913), and Jackson (1913) that the adrenal cortex of the female rat is larger than that of the male, and the male cortex of various animals increases in size after castration according to numerous observers (cited by Andersen and Kennedy, 1933). This size increase in the castrated male mouse is associated with the hypertrophy of the X zone (Howard, 1927, 1930). These reactions in the adrenal, however, probably represent effects of the sex hormones on the adrenal, and do not imply that conversely the cortical hormone might specifically affect the reproductive system.

Current clinical opinion holds that hypertrophic adrenals or adrenal tumors are frequently a causative factor in virilism in the female, pubertas praecox, or pseudohermaphroditism (Bullock and Sequeira, 1905; Glynn, 1912; Holmes, 1924; Collett, 1924; Healy and Guy, 1931; Broster and Hill, 1932; and others). Our findings lend no support to these views in so far as they imply that tumors act by increasing the body supply of the hormone of the tumor tissue, since we have not been able to cause any change in the reproductive system. The possibility remains that with doses a hundred times as great one might be able to produce sexual effects, since some cortical tumors, as in the case of Gordon Holmes, are several hundred times the weight of the normal adrenal. We have been unable as yet to adequately test this possibility because of the tremendous amounts of material required. However, our results clearly indicate that a moderate excess of cortical

extract, up to about seventeen times the body maintenance requirements, can lead to no interference with reproductive activity. Hence moderate cortico-adrenal enlargement or hyperfunction should not be regarded as in itself the cause of intersexuality.

In attempting to understand the causes of developmental abnormalities of the reproductive system, it is well to consider the biological significance of intersexuality, which has recently been considerably elucidated by genetic and cytological studies. As formulated by Bridges (1932) intersexes in *Drosophila* are characterized by a ratio of sex chromosomes to autosomes which is intermediate between the ratios characteristic for the normal male or female. Individuals bearing these intermediate chromosome ratios present various abnormalities, particularly in sexual characteristics which show all degrees of variation between male and female. Similar factors are probably operative in the production of intersexes in the human. Since the abnormal chromosome ratio, if gametogenic, will occur in every cell in the body, it manifests itself in other than sexual organs. Hence an adrenal enlargement may quite possibly be a genetic or even a specific functional *consequence* of intersexuality, rather than a cause of this condition.

According to Bullock and Sequeira (1905) and others, adrenal tumors are frequently associated with precocious sexual maturity, but on the other hand these authors list a series of cases of hypernephroma, and adrenal carcinoma and sarcoma in children, which were not associated with precocious maturity or manifest intersexuality. The absence of any demonstrable effect of the adrenal cortical hormone on the onset of sexual maturity in rats indicates that the cause of this condition is not a hyperfunction of the adrenal. This conception is in harmony with the clinical findings that adrenal tumors may occur without precocity, and that precocity may occur without adrenal tumors (Blumer, 1929). Hence the undoubtedly frequent coincidence of these two conditions must be interpreted as due to a common constitutional cause, having its basis in genetical or embryological factors or in a generalized developmental disorder of the individual.

In the type of case in which a "masculinization" of a "previously normal" adult woman is reported as taking place following the development of a cortical tumor, the symptoms of so-called masculinization (Rowntree and Ball, 1933) are often confined to hirsutism, amenorrhea, and leanness. Although on first thought this combination of symptoms suggests a masculinization, alternative explanations are fully as justifiable. The question of hirsutism has been thoroughly discussed by Danforth (1925) who has collected four cases of luxuriantly bearded women who had each borne children. Hence it is obvious that extreme development of facial hair may occur without appreciable abnormality of the female reproductive system. Consequently it is erroneous to consider hypertrichosis as an indication of a

suppression of the normal ovarian activity. Danforth reports that removal of the ovaries in thirty-two women was not followed by any abnormally excessive hair growth. The degree of hirsutism manifested in any individual tends to increase gradually with increasing age, and it may be increased in pregnancy, in acromegaly, or in the presence of non-adrenal neoplastic disease. Hirsutism may be present without adrenal or other demonstrable localized pathology, as shown also by Rowntree and Ball (1933) who report two cases of marked hirsutism in women, in which surgical exploration did not disclose abnormality of the suprarenal glands or abdominal organs, and in one case histological examination of the adrenal showed it to be normal. Hence as a symptom of either masculinization or gross adrenal disease hirsutism is not reliable. In this type of case, the improvement in general condition and resumption of menstruation following the successful removal of neoplastic tissue need not be interpreted as evidence of increased "femininity."

In those rarer cases of adrenal tumor in adult women in which there is also present modification of the genitalia in the masculine direction (Holmes, 1924; Crosbie and Smith, 1928) it is most probable that this is due to a latent or previously unrecognized and progressively developing constitutional tendency to intersexuality, which as a result of genetic factors is often manifested with a constitutional tendency to adrenal tumor. In such cases the return to the normal morphology of the genitalia following ablation of the tumor is rarely reported, so that when it is reported (Holmes, 1924) one suspects either that the changes in the genitalia may have been more apparent than real or else that the regression was not primarily caused by the ablation of the tumor.

SUMMARY

A moderate excess of the hormone of the adrenal cortex has no appreciable influence on the size of the gonads, the oestrous cycle, or the course of gestation in the rat. Contradictory previous results of others are attributed either to the presence of toxic impurities in the extracts used, or to natural variability among experimental animals.

The growth of normal young rats is not appreciably influenced by a moderate excess of the hormone of the adrenal cortex.

No causal relation of the adrenal cortex to intersexuality has been detected with the methods employed. The frequent but not invariable coincidence of intersexuality or precocious sexual maturity with adrenal cortical tumor is probably a result of a common inducing factor.

REFERENCES

ANDERSEN, D. H. AND H. S. KENNEDY. 1933. Journ. Physiol., **79**, 1.
ATWELL, W. J. 1932. Proc. Soc. Exper. Biol. and Med., **29**, 1259.
BACKMANN, E. L. 1921. Pflüger's Arch., **189**, 261.

Blumer, G. 1929. Bedside diagnosis. III. Philadelphia.
Bridges, C. B. 1932. Chapter in Sex and the Internal Secretions. Williams & Wilkins, Baltimore.
Britton, S. W. and R. Kline. 1933. This Journal, **105**, 12.
Broster, L. R. and H. G. Hill. 1932. Brit. Journ. Surg., **19**, 557.
Bullock, W. and J. H. Sequeira. 1905. Trans. Path. Soc. London, **56**, 189.
Claus, P. E. 1931. Physiol. Zool., **4**, 36.
Cleghorn, R. A. 1932a. Journ. Physiol., **75**, 413.
1932b. Journ. Physiol., **76**, 193.
Collett, A. 1924. Amer. Journ. Dis. Child., **27**, 204.
Connor, C. L. 1931. Proc. Soc. Exper. Biol. and Med., **29**, 131.
Corey, E. L. and S. W. Britton. 1931. This Journal, **99**, 33.
Crosbie, A. H. and S. W. Smith. 1928. Journ. Urol., **19**, 241.
Danforth, C. H. 1925. Arch. Dermat. and Syphil., **19**, 195, 380.
Deanesley, R. 1928. Proc. Roy. Soc. Ser. B., **103**, 523.
Donaldson, J. C. 1919. Amer. Journ. Anat., **25**, 291.
Eagle, E. 1933. Proc. Soc. Exper. Biol. and Med., **30**, 1094.
Firor, W. M. and A. Grollman. 1933. This Journal, **103**, 686.
Freed, S. C., B. Brownfield and H. M. Evans. 1931. Proc. Soc. Exper. Biol. and Med., **29**, 1.
Gaunt, R. and W. M. Parkins, 1932. This Journal, **103**, 511.
Glynn, E. E. 1912. Quart. Journ. Med., **5**, 157.
Grollman, A. and W. M. Firor. 1933a. Proc. Soc. Exper. Biol. and Med., **30**, 669.
1933b. Journ. Biol. Chem., **100**, 429.
Hatai, S. 1913. Amer. Journ. Anat., **15**, 87.
Healy, C. E. and C. C. Guy. 1931. Arch. Path., **12**, 543.
Holmes, G. 1924. Quart. Journ. Med., **18**, 143.
Howard, E. 1927. Amer. Journ. Anat., **40**, 251.
1930. Anat. Rec., **46**, 93.
Jackson, C. M. 1913. Amer. Journ. Anat., **15**, 1.
Jaffe, R. and Ranssweiller. 1926. Frankf. Zeitschr. Path., **33**, 458.
Kaplan, S. 1932. Endokrinol., **11**, 321.
Martin, S. J. 1932. This Journal, **100**, 180.
Migliavacca, A. 1933. Arch. internat. de Physiol., **36**, 137.
Müller, C. 1931. Endokrinol., **8**, 5.
Rowntree, L. G. and R. G. Ball. 1033. Endocrinol., **17**, 263.

A COMPARISON OF INTRAGASTRIC AND DUODENAL FACTORS IN LOWERING THE ACIDITY OF GASTRIC CONTENTS

CHARLES M. WILHELMJ, IRWIN NEIGUS AND FREDERICK C. HILL

From the Departments of Physiology and Experimental Surgery, Creighton University School of Medicine, Omaha, Nebraska

Received for publication October 5, 1933

In a previous communication (1933) we have shown that the presence of hydrochloric or other acids in the intact stomach or fundic pouch does not inhibit the secretion of acid by the fundic cells. The present report deals with a comparison of duodenal and intragastric factors in lowering the acidity of gastric contents. A brief review of the literature shows that there is considerable divergence of opinion on this point.

In 1915 Boldyreff published his theory of the regulation of gastric acidity in which neutralization of acid by regurgitated pancreatic juice was the essential factor. In the same year Spencer, Meyer, Rehfuss and Hawk published data which supported and amplified this theory. Since that time evidence of a similar nature has been published by Hicks and Vischer (1925), Bolton and Goodhart (1922), Elman (1928), Olch (1928), Olch and Elman (1928), and Elman and Eckert (1933). All of these investigators emphasized the importance of neutralization by alkaline pancreatic juice. In 1924 Baird, Campbell and Hern emphasized the importance of intragastric neutralization of acid but admitted that regurgitation of duodenal contents was often a factor of great importance. Yesko (1928), McCann (1929), and Shay, Katz, and Schloss (1932) deny that the regurgitation of duodenal contents is a factor of any importance in the regulation of gastric acidity and express the opinion that the regulation is entirely by intragastric mechanisms. McCann emphasizes intragastric neutralization while Shay, Katz and Schloss believe that hydrochloric acid is absorbed by the stomach.

Methods and procedure. The general methods and calculations employed have been given in detail in the first paper of this series (1933) and need not be repeated. We have employed both the fractional and block methods of gastric analysis but have found the block method to be more satisfactory.

In the present studies we have routinely employed two-thirds normal sulfuric acid and 10 per cent sodium tungstate, as previously described, to remove mucus, bile or other substances which might make the solutions

turbid and render the colorimetric determination of the phenol red inaccurate. A series of control experiments was performed in which egg albumin solutions were added to hydrochloric acid solutions containing phenol red and in these experiments we were able to show that it is possible to precipitate much larger amounts of protein than were ever encountered in gastric or duodenal samples without loss of the dye by adsorption. It is

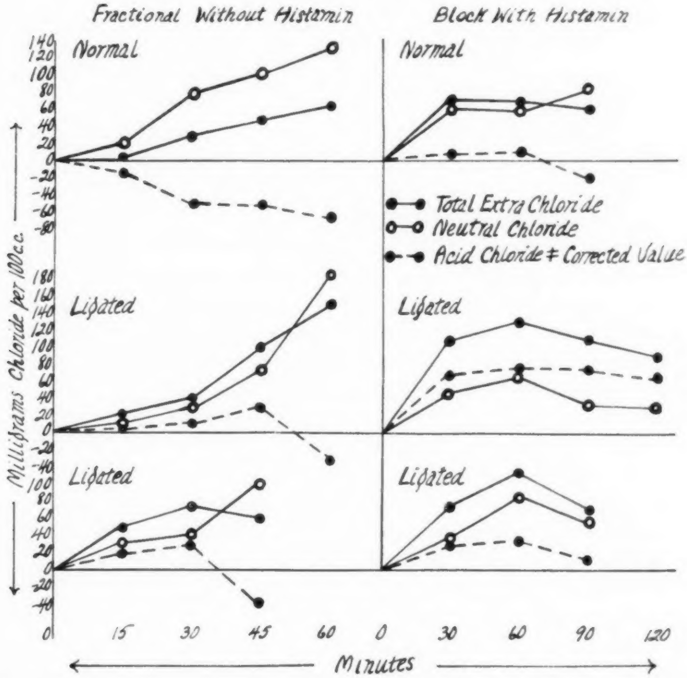


Fig. 1. Shows the response of the same dog before and after ligation of the pancreatic and bile ducts. The acid solution introduced into the stomach contained 348 mgm. of acid chloride per 100 cc. The results are given as milligrams per cent. The zero line represents the correction for dilution and values below this line indicate neutralization.

necessary to emphasize that the samples should be centrifuged at high speed for about 10 minutes after the addition of the sulfuric acid and sodium tungstate and again after the addition of alkali. It frequently happens, especially in duodenal samples, that there is a diffuse turbidity after the addition of sulfuric acid and sodium tungstate which cannot be removed by centrifuging; after the addition of alkali, however, this material is precipitated completely. By observing the above precautions we have

been able to remove all turbidity and bile and to obtain absolutely clear samples in which the determination of phenol red is very satisfactory.

In the studies on the intact stomach the bile content was determined by employing a standardized Pettenkofer reaction. The same quantity of reagents, the same time, etc., were always employed and the results read independently by two parties. A system of notation was used in which the amount of bile was recorded as ranging from zero to four plus. While the method is admittedly crude and has shown individual variations, for instance, the finding of more evidence of regurgitation with a 3 plus than with a 4 plus, the general average results have seemed quite satisfactory.

a. *The effect of ligation of the pancreatic and bile ducts.* One of the dogs used in these studies (dog 1 in paper I) showed considerable evidence of regurgitation of duodenal contents and it was decided to investigate the effect of ligation of the pancreatic and bile ducts. Approximately twenty complete experiments were obtained before ligation and eight after ligation. Figure 1 shows a group of fractional experiments without histamin and block experiments with histamin before and after ligation. In the fractional experiments, before ligation, there was a very prompt and progressive decrease in the acid chloride below the level corrected for dilution; since we have never obtained any evidence that hydrochloric acid is absorbed from the stomach, this decrease must represent neutralization. As the acid chloride decreased the neutral chloride increased but the increase of the neutral chloride was greater than the decrease in acid chloride; the difference representing either neutral chloride which entered the stomach with the duodenal and pyloric secretions or hydrochloric acid which was secreted by the stomach and subsequently neutralized. After ligation the acid chloride remained above the level corrected for dilution, showing that a small amount of acid was being secreted by the stomach but was not being neutralized. After 45 or 60 minutes the acid chloride dropped below the level corrected for dilution, indicating that neutralization had occurred by this time, however, the stomach was nearly empty so that the total amount of acid neutralized was actually quite small. After ligation there was a marked increase of neutral chloride and since the amount of neutralization was quite small (not sufficient to completely neutralize even a small amount of acid secreted by the stomach) the greater part of the neutral chloride was probably brought in as a constituent of pyloric secretion or regurgitated succus entericus. These experiments show that reduction of the acidity of gastric contents was definitely interfered with by ligation of the pancreatic ducts but also show that there was some reduction which became evident when the volume of acid solution in the stomach was very small.

The block experiments with histamin stimulation show similar changes.

Before ligation large amounts of duodenal contents were regurgitated so that nearly all of the secreted acid was neutralized and appeared as neutral chloride. After ligation the secreted acid was not neutralized and remained as extra acid chloride. The neutral chloride was quite high after ligation showing that considerable quantities of neutral chloride were coming into the stomach with the pyloric secretion and with the regurgitated succus entericus.

These experiments were repeated on two other dogs. In one the results were quite similar to those shown in figure 1 while in the other there was much less change after ligation.

There is one possible fallacy in the interpretation of the above experiments. Fauley and Ivy (1929) have shown that ligation of the pancreatic ducts causes a marked hypersecretion of acid in Pavlov pouches. If this occurred in our dogs the higher acid values after ligation might be due not only to decreased neutralization but also to increased secretion. In general the extra total chloride values were considerably higher after ligation, especially in the block experiments with histamin, indicating that this hypersecretion may have occurred. Because of this fact it is quite possible that these experiments falsely magnify the rôle of pancreatic juice in lowering the acidity of gastric contents.

b. *Experiments with pyloric pouches.* Any intragastric neutralization which occurs must be due to the faintly alkaline mucus secreted by the stomach. It is quite probable that the mucus secreted by the fundus is similar to that secreted by the pyloric region. A study of the neutralizing power of pyloric pouches will therefore give a general idea of the extent of intragastric neutralization.

Three pyloric pouches were prepared according to the method of Goldberg and Mann (1932). The hydrochloric acid solution used contained 348 mgm. of acid chloride per 100 cc. Four milligrams of phenol red were added to 750 cc. of acid solution. The acid solution was not simply placed in the pouch and allowed to remain there during the experimental period, it was rhythmically introduced into the pouch and immediately withdrawn at the rate of 4 to 5 times each minute for a period of one-half hour. The pouch was closed by a rubber stopper through which the catheter passed so that no solution was lost. It was found that the pouches had a very definite periodic phase of contraction and relaxation and the introduction and withdrawal of the acid solution was made to correspond to this. The acid solution remained in the pouch for only about 12 to 15 seconds at a time, thus it served to stimulate the pouch and became intimately mixed with the secretions but there was little danger of any absorption taking place. Before starting each experiment the pouch was always lavaged with several changes of acid solution in order to remove any secretion which was in the pouch. One milligram of histamin was

TABLE 1

Shows the response of pyloric pouches when a hydrochloric acid solution containing 348 mgm. of acid chloride per 100 cc. was instilled in and out of the pouch at the rate of 4 to 5 times per minute for one half hour period. The amount of the various chloride fractions in the fluid are given both as milligrams per cent and as the amount actually present in the fluid.

DOG	TIME AFTER HISTAMIN, HOURS	P.S.P. PER CENT	CHLORIDE OF ORIGINAL SOLUTION CORRECTED FOR DILUTION	MILLIGRAMS PER 100 CC.					VOLUME OF SAMPLE, CC.	AMOUNT ACTUALLY PRESENT IN SAMPLE MILLIGRAMS				CHLORIDE OF SECRETION, MGM. PER 100 CC.	Chloride of neutralized acid / cc. of secretion	Neutral chloride / cc. of secretion
				Total chloride	Neutral chloride	Total minus neutral	Extra chloride	Acid chloride ± corrected value		Extra chloride	Neutral chloride	Acid chloride ± corrected value	Amount of secretion, cc.			
1	½	79	275	351	123	228	76	−47	20	15	25	−9	4	362	2.2	5.9 (4.6)
	1	84	292	360	81	279	68	−13	20	14	16	−3	3	425	0.8	5.1 (4.7)
	1½	85	296	362	79	283	66	−13	19	13	15	−3	3	440	0.9	5.2 (4.3)
1	½	83	289	352	92	260	63	−29	19	12	18	−6	3	370	1.7	5.4 (4.6)
	1	81	282	357	99	258	75	−24	20	15	20	−5	4	395	1.3	5.2 (4.1)
	1½	81	282	352	98	254	70	−28	21	15	21	−6	4	370	1.5	5.2 (4.2)
1	½	78	272	371	139	232	99	−40	17	17	24	−7	4	450	1.8	6.3 (4.6)
	1	75	262	366	134	232	104	−30	17	18	23	−5	4	416	1.2	5.4 (4.8)
	1½	71	248	366	141	225	118	−23	18	20	25	−4	5	407	1.8	4.9 (4.4)
2	½	84	298	350	61	289	52	−9	19	10	12	−2	3	325	0.6	3.8 (3.7)
	1	82	315	363	44	319	48	+4	19	9	8	+0.8	2	436		4.0 (4.0)
	1½	92	326	354	41	313	28	−13	19	5	8	−3.0	2	350	1.6	5.1 (3.1)
2	½	91	316	343	67	276	27	−40	20	5	13	−8.0	2	300	4.4	7.4 (3.7)
	1	86	299	355	60	295	56	−4	20	11	12	−0.8	3	400	0.3	4.3 (3.7)
	1½	89	310	345	48	297	35	−13	19	7	9	−3.0	2	318	1.2	4.4 (3.8)

3	½	61	212	352	211	141	140	−71	18	25	38	−13	7	359	1.8	5.4 (4.1)
	1	70	244	349	143	206	105	−38	18	19	26	−7	5	350	1.3	4.8 (4.2)
	1½	70	244	353	143	210	109	−34	18	20	26	−6	5	363	1.1	4.8 (4.3)
3	½	67	233	352	162	190	119	−43	14	17	23	−6	5	361	1.3	4.6 (3.7)
	1	74	256	349	121	228	93	−28	19	18	23	−5	5	358	1.1	4.6 (3.8)
	1½	80	278	346	126	220	68	−58	14	10	18	−8	3	340	2.9	6.3 (4.0)
Average										15	19	−5	4	376	1.5	5.2 (4.1)

injected intramuscularly just before starting the experiment in order to make these experiments comparable to the experiments on the whole stomach. Three one-half hour experiments were performed after the injection of histamin. Twenty one half hour experiments were performed on three pouches. The results are shown in table 1. The results are given both in terms of concentration (mgm. per 100 cc.) and as the actual amount of the various chloride fractions. Since these pouches represent the entire pyloric region, the latter figures show approximately how much change could be brought about by the pyloric region in the whole stomach. In paper I (1933) we have pointed out the obvious fallacies in interpretation which may occur when the various chloride fractions in small volumes are expressed in terms of milligrams per cent.

RESULTS (table 1). 1. *The chloride content of the secretion* ranged from 300 to 450 mgm. per 100 cc. and averaged 376 mgm. This value is lower than the value reported by Ivy and Oyama (1921) (632 mgm. per 100 cc.) and that reported by Gamble and McIver (1928) (561 mgm. per 100 cc.). It is also lower than a value obtained by us in a sample of pure secretion removed from one of the pouches before starting an experiment. It is quite likely that concentration may occur when the secretion is allowed to accumulate in the pouch or it may be that the secretion provoked by acid stimulation is more dilute than the resting secretion. At any rate the value obtained by acid stimulation is the one of particular interest in the present problem.

2. *The extra chloride* in the form of neutral chloride which was added to the acid solution by the pyloric pouches during the half-hour period averaged 15 mgm.

3. *The total neutral chloride* in the samples removed from the pouches is composed of the neutral chloride added by the pyloric secretions plus the neutral chloride resulting from neutralization of hydrochloric acid. The average value for all experiments was 19 mgm.

4. *The chloride of the acid neutralized by each cubic centimeter of pyloric secretion* shows a maximum variation of from 0.3 to 4.4 mgm. in individual experiments and averages 1.5 mgm. From this it can be calculated that the alkalinity of pyloric secretion averages approximately 0.04 normal. Bolton and Goodhart (1933) found that the alkalinity of gastric mucus averaged approximately 0.04 normal, while Gamble and McIver (1928) found that the base in excess of chloride in pyloric secretion was approximately 11 cc. of tenth normal per 100 cc.

5. *The total amount of pyloric secretion* for the half-hour period varied from 2 to 7 cc. and averaged 4 cc. Ivy and Oyama (1921) found that the only stimulant of pyloric secretion was the presence of acid in the pouch. Since the concentration of the acid solution was reduced both by dilution and neutralization during the half-hour period, it may not have exerted its

full stimulating effect. Two experiments were performed on pouch 1 in which fresh acid solution was used every 7 to 8 minutes during the half-hour period and the secretion in these four samples added to represent the total secretion for the half-hour period. Under these conditions the secretion was definitely increased in one instance to 9 cc. and in another to 14 cc. Thus if the acid solutions in the whole stomach remain at a high level it is possible for the pyloric secretion to approximate 14 cc. for a half-hour period. Ivy and Oyama (1921) found the basal secretion to average 3 cc. per hour and to be increased from 2 to 3 times by tenth normal acid.

6. *The ratio of total neutral chloride to the cubic centimeter of secretion* shows how much neutral chloride can be related to each cubic centimeter of secretion; this includes the neutral chloride content of each cubic centimeter of secretion plus the neutral chloride resulting from the neutralization of acid by each cubic centimeter of secretion. This ratio averages 5.2 with variations from 3.8 to 7.4. The significance of this ratio will be discussed later.

c. *Experiments on duodenal pouches.* The fluid which normally regurgitates from the duodenum into the stomach is a mixture of bile, pancreatic juice and succus entericus. Pure pancreatic juice possibly never regurgitates into the stomach. The composition of mixed duodenal secretions was studied in duodenal pouches.

The total loss of pancreatic juice for more than a few days is invariably fatal. After pancreatic juice has been lost for several days, the juice becomes very dilute and its alkalinity definitely reduced. It was therefore necessary to prepare duodenal pouches which could be used immediately and which were completely healed at the time of the experiment. A method was developed in which the pouches were made by a two stage operation. After the first stage the duodenal contents still drained into the intestine and the animal would remain in normal condition indefinitely. The pouch was completed at the second stage which was a short operation, usually requiring only 20 to 25 minutes. The completed pouch was entirely healed except the end which was brought to the outside. Before using this was lightly cauterized so that no blood or serum contaminated the contents. The pouches included the upper 6 or 8 inches of the duodenum with the entrance of the bile and pancreatic ducts. The second stage was performed in the late afternoon. After the operation 300 to 500 cc. of 2 per cent sodium bicarbonate in physiological saline were injected subcutaneously. The next morning the animals were in good condition and the first series of experiments was performed. Following these experiments the animals were again injected with from 300 to 500 cc. of bicarbonate and saline subcutaneously. The following morning the animals were still in good condition and the second series of experiments was performed. No experiments were performed after the second day.

The manner of performing the experiments was very similar to that used on the pyloric pouches. Thirty cubic centimeters of acid solution were injected into and immediately withdrawn from the pouch with a syringe and soft rubber catheter, the end of the pouch being closed with a rubber stopper. Care was taken to avoid distention of the pouch since this caused severe vomiting. The pouch was lavaged with acid solution before starting the experiment. One milligram of histamin was injected intramuscularly. The acid solution was run in and immediately withdrawn from the pouch about once per minute for a period of 30 minutes. Three or four half-hour samples were obtained after the injection of histamin. The acid solution presumably stimulated the secretion of secretin and was intimately mixed with the duodenal secretions but was not left in the pouch long enough for any to be absorbed. At irregular intervals the acid solution would show marked effervesence as it was drawn back into the syringe, suggesting that a spurt of pancreatic juice had been delivered into the pouch. The samples were always lightly bile stained but at times a profuse staining with dark bile would occur suddenly as if the gall bladder had emptied. These observations indicate that the pouches were responding in a normal manner. Nineteen half-hour experiments were performed on three pouches.

Results (table 2). 1. *The chloride content* of the secretion averaged 310 mgm. per 100 cc. which is slightly lower than pyloric secretion.

2. *The chloride of the acid neutralized by each cubic centimeter of duodenal secretion* varied from 0.09 to 2.8 mgm. in individual experiments and averaged 1.5 mgm. for all experiments. This shows an average alkalinity of approximately 0.04 normal. The variations are probably dependent upon the amount of pancreatic juice added. The highest alkalinity was 0.08 normal. The average value is the same as the average obtained for pyloric secretion. The alkalinity of pure pancreatic juice according to Starling is approximately 0.1 normal; Gamble and McIver (1928) found it to vary from 0.06 to 0.096 normal; Hartman and Elman (1929) found it approximately 0.1 normal; Elman and McCaughan (1927) approximately 0.1 normal. Our findings indicate that in the mixed duodenal secretions the pancreatic juice was diluted by the nearly neutral bile and succus entericus, the average dilution being approximately 50 per cent.

3. *The ratio of the total neutral chloride to the cubic centimeters of duodenal secretion* averaged 4.7 and varied from 5.8 to 3.6. This ratio is lower than the ratio obtained for pyloric secretion.

4. *The total amount of duodenal secretion* for the half-hour period averaged 19 cc. and varied from 14 to 24 cc.

d. *Experiments on the intact, whole stomach.* This report is based upon 68 experiments performed upon three normal dogs. All experiments are of the block type, fractional experiments being omitted because of the greater

uncertainty in establishing base lines. A complete description of the methods and calculations has been given in a previous paper (1933). In general the method consisted in introducing a definite volume (300 to 400 cc.) of standard hydrochloric acid solution containing phenol red into the stomach and injecting 1 mgm. of histamin intramuscularly; at the end of one-half hour the stomach was completely emptied and immediately refilled with fresh acid solution. This was repeated three times thus giving three one-half hour samples after the injection of histamin.

The interpretation of these experiments is based on the following considerations: The reduction of the per cent of phenol red in the hydrochloric acid solution removed from the stomach shows the per cent of fluid which was added to the acid solution while in the stomach. This added fluid consists of the following secretions in variable proportions: Hydrochloric acid and mucus secreted by the fundic cells, pyloric secretions and regurgitated duodenal secretions. In previous studies (1933) we found that the chloride concentration of fundic secretion averaged 578 mgm. per 100 cc. and contained only small amounts of neutral chloride. The chloride concentration of the fundic secretion was found to remain constant and was not altered by the hydrogen or chloride ion concentration of the gastric contents. If the extra acid chloride in the solution removed from the stomach is divided by 5.78, the quotient will represent the cubic centimeters of hydrochloric acid secreted by the stomach which was not neutralized. The difference between this figure and the total amount of fluid added to the solution placed in the stomach constitutes what will be spoken of as "extra fluid." The "extra fluid" is composed of the following fractions: hydrochloric acid which was secreted by the fundic cells but was subsequently neutralized, pyloric secretions, and regurgitated duodenal secretions. The chloride of the acid which was secreted and subsequently neutralized is present as neutral chloride. The pyloric and regurgitated duodenal secretions are the fluids responsible for neutralization but they also contain neutral chloride. Thus it is evident that only a part of the neutral chloride represents neutralized acid. If the neutral chloride is divided by the cubic centimeters of extra fluid, the ratio will represent the amount of the neutral chloride related to each cubic centimeter of extra fluid.

According to the above interpretation the neutral chloride-extra fluid ratio in the intact whole stomach should be approximately the same as that found in duodenal and pyloric pouches. As shown in table 3, however, the ratios in the whole stomach are somewhat lower than those obtained in duodenal and pyloric pouches. A study of the manner in which the ratios are calculated shows that this difference should exist since in the whole stomach the extra fluid includes the fluid of the acid which was secreted and subsequently neutralized, while in the pouch experiments this fluid is not included, the neutral chloride simply being divided by the cubic centi-

TABLE 2

Shows the response of duodenal pouches when a hydrochloric acid solution containing 348 mgm. of acid chloride per 100 cc. was instilled in and out of the pouch at the rate of one time per minute for a period of one-half hour. The amounts of the various chloride fractions in the fluid are given both as milligrams per cent and as the amount actually present in the fluid.

DOG	TIME AFTER HISTAMIN, HOURS	P.S.P. PER CENT	CHLORIDE OF ORIGINAL SOLUTION CORRECTED FOR DILUTION	MILLIGRAMS PER 100 CC.: Total chloride	MILLIGRAMS PER 100 CC.: Neutral chloride	MILLIGRAMS PER 100 CC.: Total minus neutral	MILLIGRAMS PER 100 CC.: Extra chloride	MILLIGRAMS PER 100 CC.: Acid chloride± corrected value	VOLUME OF SAMPLE, CC.	AMOUNT ACTUALLY PRESENT IN SAMPLE MILLIGRAMS: Extra chloride	AMOUNT ACTUALLY PRESENT IN SAMPLE MILLIGRAMS: Neutral chloride	AMOUNT ACTUALLY PRESENT IN SAMPLE MILLIGRAMS: Acid chloride± corrected value	AMOUNT ACTUALLY PRESENT IN SAMPLE MILLIGRAMS: Amount of secretion, cc.	CHLORIDE OF SECRETION, MGM. PER 100 CC.	Chloride of neutralized acid / cc. of secretion	Neutral chloride / cc. of secretion
1	½	55	192	319	172	147	127	−45	48	61	83	−22	22	282	1.0	3.8 (3.2)
	1	65	226	317	152	165	91	−61	51	46	78	−31	18	260	1.7	4.3 (3.4)
	1½	65	226	306	162	144	80	−82	48	38	78	−39	17	230	2.3	4.6 (3.3)
1	½	62	216	319	187	132	103	−84	52	54	97	−43	20	271	2.2	4.9 (3.5)
	1	69	240	323	170	153	83	−87	52	43	88	−44	16	268	2.8	5.5 (3.7)
	1½	63	220	314	188	126	94	−94	51	49	96	−49	19	254	2.5	5.8 (3.6)
2	½	48	167	336	206	130	169	−37	41	69	85	−15	21	325	0.71	4.0 (3.5)
	1	61	212	335	186	149	123	−63	42	52	78	−27	16	315	1.6	4.8 (3.7)
	1½	58	202	330	191	139	128	−63	41	53	78	−26	17	305	1.5	4.6 (3.7)
2	½	54	188	350	166	184	162	− 4	42	68	70	−1.7	19	352	0.09	3.6 (3.7)
	1	58	202	340	177	163	138	−39	40	55	71	−16	17	329	0.93	4.2 (3.6)
	1½	65	226	348	165	183	122	−43	41	50	68	−18	14	349	1.2	5.0 (4.0)
	2	60	209	341	165	176	132	−33	38	50	63	−13	15	330	0.83	4.1 (3.7)

3	½	57	198	334	188	146	136	−52	43	59	81	−22	19	316	1.2	4.4 (3.5)
	1	50	174	356	214	142	182	−32	46	84	98	−15	23	364	0.64	4.3 (3.8)
	1½	53	185	349	211	138	164	−47	46	75	97	−22	22	345	1.0	4.5 (3.7)
	½	56	195	349	229	120	154	−75	51	79	117	−38	22	327	1.7	5.2 (4.2)
	1	58	202	342	231	111	140	−91	49	69	113	−45	21	333	2.2	5.5 (3.9)
	1½	46	160	342	302	40	182	−120	45	82	136	−54	24	336	2.2	5.8 (4.1)
Average										60	88	−32	19	310	1.5	4.7 (3.7)

TABLE 3

An analysis of experiments on the intact stomach using standard hydrochloric acid solutions. Stomach stimulated with histamin. Calculations are given in detail on dog 1 while important averages of a similar number of experiments are given for dogs 2 and 3. The various calculations are based on the total quantity and not on per cent.

CHLORIDE OF ACID SOLUTION	P.S.P. PER CENT	ACID CHLORIDE± CORRECTED VALUE	NEUTRAL CHLORIDE	TOTAL FLUID ENTERING STOMACH—CC.	FLUID FROM EXTRA ACID	EXTRA FLUID, CC.	Neutral chloride / Extra fluid	VOLUME	BILE	REMARKS
348 mgm. chloride per 100 cc.	85	+173	103	53	30	23	4.5	345	+	
	83	+276	152	76	48	28	5.4	446	+	
	86	+169	141	56	29	27	5.2	402	+	
348 mgm. chloride per 100 cc.	86	+110	166	57	19	38	4.3	406	+++	
	86	+206	101	57	36	21	4.8	404	0	
	81	−137	161	46	—	46	3.5	240	++++	Vomited
348 mgm. chloride per 100 cc.	84	+225	113	69	39	30	3.8	432	0	
	77	+316	156	94	55	39	4.0	410	VF+	
	83	−27	66	15	—	15	4.4	90	++++	Vomited
348 mgm. chloride per 100 cc.	83	+219	112	73	38	25	3.2	430	—	
	81	+301	87	75	52	23	3.8	396	0	
	81	−14	318	77	—	77	4.1	406	+++	
179 mgm. chloride per 100 cc.	83	+148	123	60	26	34	3.6	352	+	
	78	+348	111	84	61	23	4.8	382	VF+	
	83	+215	78	49	37	12	6.5	290	VF+	
179 mgm. chloride per 100 cc.	87	+210	84	55	36	19	4.4	420	0	
	78	+271	167	78	47	31	5.2	356	+	
	87	+67	134	40	12	28	4.8	304	+	

79 mgm. chloride per 100 cc.	87	+164	80	44	28	16	5.0	334	0	
	81	+249	96	55	43	12	8.0	289	VF+	
	87	+30	82	23	5	18	4.5	174	+	
79 mgm. chloride per 100 cc.	82	+271	97	73	47	26	3.7	403	0	
	73	+266	49	67	50	17	2.9	246	0	
	82	+100	76	40	17	23	3.0	222	VF+	
Average						27.1	4.3			
Average dog 2						27.4	4.0			
Average dog 3						16.2	5.0			
Average for 68 experiments						24	4.4			

meters of fluid secreted by the pouch. The ratios in the pouches can be made to correspond to those in the whole stomach by assuming that the acid neutralized contained 578 mgm. of chloride per 100 cc. and adding the fluid of this neutralized acid to the fluid secreted by the pouch. When this is done the ratios in the pouches are lowered and are in fair agreement with the ratio obtained in the whole stomach. The corrected ratios are shown in parenthesis in tables 1 and 2. The agreement between the ratios is sufficiently good to justify the conclusion that the extra fluid and neutral chloride, in the whole stomach experiments, are composed of varying proportions of the fluid and neutral chloride obtained in pyloric and duodenal pouches.

Since the ratio in pyloric secretion is slightly higher than that in duodenal secretion it should follow that, in the whole stomach, the highest ratio should be found when there is least evidence of duodenal regurgitation and that it should be lowered as more duodenal fluid enters the stomach.

TABLE 4

Shows the relationship between the amount of bile, extra fluid, and the neutral chloride—extra fluid ratio. Average of 68 experiments on three normal dogs. The extra fluid is calculated as the total quantity present and not in terms of cubic centimeters per cent.

BILE	EXTRA FLUID	$\frac{\text{NEUTRAL CHLORIDE}}{\text{EXTRA FLUID}}$
0	17	4.6
+	23	4.6
++	25	4.2
+++	44	4.2
++++	32	4.1

Table 4 shows that this does occur. In the averages for the individual dogs the same tendencies are evident. In dog 2 the average ratio was 4.0 and large amounts of duodenal fluid were usually regurgitated; in dog 3 the average ratio was 5.0 and there were usually only traces of bile present; dog 1 was intermediate.

In table 3 in which the calculations are based on the total quantity of fluid removed from the stomach each half-hour, it is seen that the total amount of extra fluid may be of considerable magnitude. When the amount of extra fluid was compared with the amount of bile present, it was found that in general these two factors increased together (table 4). The average extra fluid in all experiments which contained no bile was 17 cc. In figure 2 the relationship between neutral chloride, extra fluid, bile content and uncorrected acid deficit (which includes acid deficit due both to neutralization and to dilution) is shown. The results are expressed as amounts per 100 cc. It is clear that these four factors increase together and are definitely related.

Discussion. The experiments with pyloric and duodenal pouches show that the alkalinity of duodenal and pyloric secretions is of approximately the same magnitude, so that if equal quantities were available there would be little difference in their ability to reduce gastric acidity. The experiments with pyloric pouches appear to indicate, however, that the amount of pyloric secretion is rather limited in amount. From the experiments on the intact stomach it appears that during stimulation of the stomach with histamin, the maximal secretion rate of neutralizing or diluting fluid of intragastric origin is approximately 17 cc. per half-hour. This figure seems somewhat high in view of the fact that the pyloric pouch experiments

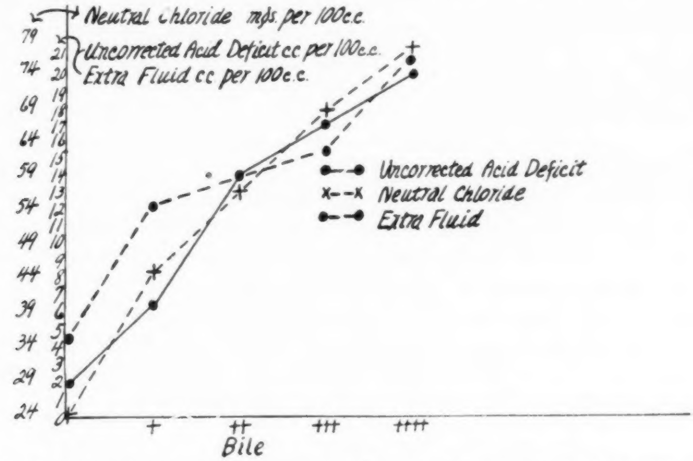


Fig. 2. An analysis of experiments on 3 normal dogs showing the relationship between the total acid deficit (including both neutralization and dilution), neutral chloride, extra fluid and bile content of the samples removed from the stomach. Results are given as the amount per 100 cc. of fluid removed from the stomach. The acid solution used contained 348 mgm. of acid chloride per 100 cc.

averaged only 4 cc. of secretion and that even when special experiments were performed with repeated fresh acid solutions we were able to obtain only 9 and 14 cc. of secretion. The higher figure obtained in the intact stomach may be due to mucus secreted by the fundic region or it may indicate that it is possible for small amounts of duodenal contents with practically no bile to regurgitate into the stomach, an interesting possibility in view of the recent observation of Ivy (1933). The total amount of neutralizing or diluting fluid entering the stomach during the half-hour period frequently amounted to between 30 and 40 cc. The amounts over 17 cc. were quite likely of duodenal origin as shown by the concomitant increase in bile content. It should be stated here that experiments were performed

to show that the entrance of bile into the stomach was not brought about artificially by manipulation with the stomach tube. In one dog a piece of ileum was attached to the stomach and brought out through a stab wound. Samples of gastric contents could be obtained with a soft rubber catheter without the use of suction; even under these conditions bile was found present in gastric contents.

A second point of interest is the significance and origin of the neutral chloride present in gastric contents. It has been customary to assume that the entire neutral chloride fraction arises from the neutralization of hydrochloric acid and it has frequently been used as an accurate index of neutralization. The present studies show that, on an average, out of every 5 mgm. of neutral chloride approximately only 1.5 to 2 mgm. are actually from neutralized acid, the remainder having entered the stomach as sodium chloride with the pyloric and regurgitated duodenal secretions.

A third point deserving special emphasis is concerned with the regurgitation of succus entericus after ligation of the pancreatic duct (fig. 1). Before ligation the amount of extra fluid entering the stomach averaged 14 cc. per 100 cc.; after ligation the average was somewhat over 13 cc. per 100 cc. Large amounts of neutral chloride were present after ligation. From our analysis and from figures reported in the literature it appears that the sodium chloride content of succus entericus and pancreatic juice are approximately the same, hence it would not be expected that the amount of neutral chloride in the gastric contents would be materially changed following ligation. The neutral chloride extra fluid ratio, however, dropped from an average of 4.0 before ligation to 3.1 after ligation.

SUMMARY

1. Studies before and after ligation of the bile and pancreatic ducts showed that following ligation, acid solutions placed in the stomach were reduced in acidity less rapidly and less completely than before ligation. Reduction in acidity became evident only when the volume of solution remaining in the stomach was very small.

2. In isolated pyloric and duodenal pouches it was found that the alkalinity of the pyloric and duodenal secretions was about the same, the average value being approximately 0.04 normal. The chloride content of pyloric secretion averaged 376 mgm. per 100 cc. while that of duodenal secretions averaged 310 mgm. per 100 cc. The ratio of neutral chloride to cubic centimeters of secretion was found to average 5.2 in pyloric and 4.7 in duodenal secretions. A correction of these ratios reduced them to 4.1 and 3.7 respectively. The total amount of secretion by pyloric pouches when filled with tenth normal hydrochloric acid averaged 4 cc. for half-hour periods and under certain conditions the amount could be raised to about 9 or 14 cc. Duodenal pouches secreted larger amounts, the average quantity for half-hour periods being 19 cc.

3. In experiments on the intact normal stomach filled with tenth normal hydrochloric acid and stimulated with histamin it was possible to separate the total fluid entering the stomach into two parts, first, secreted hydrochloric acid which was not neutralized and a second part which is called the "extra fluid" which consists of neutralized hydrochloric acid, pyloric secretions and regurgitated duodenal secretions. The ratio of the neutral chloride to the cubic centimeters of extra fluid averaged 4.4 in 68 experiments while the value in individual dogs ranged from 4.0 to 5.0. These ratios are very similar to those obtained in pyloric and duodenal pouches and seem to justify the conclusion that the ratios in the whole stomach are similar in their significance to those in the pouches.

4. In the whole stomach there was a direct relationship between the amount of extra fluid, neutral chloride, bile and acid deficit.

5. These experiments show quite clearly that it is a mistake to consider the entire neutral chloride fraction of gastric contents as arising from neutralized hydrochloric acid.

6. Both pyloric and duodenal secretions reduce acidity more by dilution than by neutralization and the greater efficiency of regurgitated duodenal contents is due to the fact that more of it may be available.

REFERENCES

Baird, M. Mc., J. M. H. Campbell and J. R. B. Hern. 1924. Guy's Hosp. Rept., **74,** 23.

Boldyreff, W. 1915. Quart. Journ. Exp. Physiol., **8,** 1.

Bolton, C. and G. W. Goodhart. 1922. Lancet, **1,** 420.
 1933. Journ. Physiol., **77,** 287.

Bulger, H. A., C. M. Stroud and M. L. Heideman. 1928. Journ. Clin. Invest., **5,** 547.

Elman, R. and J. M. McCaughan. 1927. Journ. Exp. Med., **45,** 561.

Elman, R. 1928. Arch. Surg., **16,** 1256.

Elman, R. and C. T. Eckert. 1933. Proc. Soc. Exp. Biol. and Med., **30,** 1343.

Fauley, G. B. and A. C. Ivy. 1929. This Journal, **89,** 428.

Gamble, J. L. and M. A. McIver. 1928. Journ. Exp. Med., **48,** 837, 849.

Goldberg, S. L. 1932. Arch. Int. Med., **49,** 816.

Gorham, F. D. 1923. Journ. Amer. Med. Assn., **81,** 1738.

Hartman, A. F. and R. Elman. 1929. Journ. Exp. Med., **50,** 387.

Hicks, C. J. and J. W. Vischer. 1915. This Journal, **39,** 1.

Hollander, F. 1932. Journ. Biol. Chem., **97,** 585.

Ivy, A. C. and Y. Oyama. 1921. This Journal, **57,** 51.

Ivy, A. C. 1933. Journ. Amer. Med. Assn., **100,** 1319.

McCann, J. C. 1929. This Journal, **89,** 483.

Olch, I. Y. 1928. Arch. Surg., **16,** 124.

Olch, I. Y. and R. Elman. 1928. Proc. Soc. Exp. Biol. and Med., **25,** 184.

Spencer, W. H., G. P. Meyer, M. M. Rehfuss and P. B. Hawk. 1915. This Journal, **39,** 459.

Shay, H., A. B. Katz and E. M. Schloss. 1932. Arch. Int. Med., **50,** 605.

Starling, E. H. 1926. Principles of human physiology. Lee & Febiger.

Wilhelmj, C. M., I. Neigus and F. C. Hill. 1933. This Journal, **106,** 381.

Yesko, S. A. 1928. This Journal, **86,** 483.

THE PHYSIOLOGIC MAINTENANCE OF THE MALE SEX GLANDS

The Effect of Androtin on Hypophysectomized Rats

EUGENE L. WALSH,[1] W. KENNETH CUYLER AND D. ROY McCULLAGH

From the Cleveland Clinic, Cleveland, Ohio

Received for publication October 6, 1933

The male sex glands, particularly the prostate, have been the subject of a series of collaborative investigations in this laboratory during the past few years. It has been adequately demonstrated that a hormone (androtin) can be extracted from the urine which, when administered to castrated animals, prevents atrophy of the secondary sex glands. It seems probable that this is a testicular hormone (McCullagh, 1932). The work herein reported was undertaken as part of a study to determine whether or not this male sex hormone would maintain the function of the prostate and seminal vesicles in the absence of the pituitary gland. A preliminary report of this work has been published, and we wish now to present the final results (Walsh et al., 1933).

It is well known that atrophy of the secondary sex glands occurs after castration (Freemantle, 1897; White, 1893; Moore et al., 1928, 1929, 1930) or pituitary ablation (Smith, 1927, 1930; Richter and Wislocki, 1930). Moore and Price (1932) report that Vatna found that the prostate and seminal vesicles of rats atrophy just as rapidly after hypophysectomy as after castration. Vatna is said to have found that the testicular hormone maintained the secondary sex glands of hypophysectomized rats, and also that if atrophic changes were permitted to occur, the testicular hormone would effect a regeneration. In addition, Moore and Price have stated that the testicular hormone did not prevent degenerative changes in the testes. Moore (1931) says that the injection of the lipoid-soluble male sex hormone prepared from testes leads to testicular damage, and also that the gonadal hormones have no direct effect on the gonads of the same or opposite sex.

In this series of experiments, white rats were used. Some of these were procured on the market and some were litter mates from our stock of Wistar Institute rats. Hypophysectomy was performed by a modified retropharyngeal approach after a combination of the methods of Smith (1930) and of Thompson (1932). Pentobarbital sodium,[2] 33 mgm. per kilogram

[1] Belcher Fellow in urology.

[2] This drug was furnished for these experiments by the Abbott Company.

of body weight, was used as the anesthetic; 1/200 grain of atropine was also injected intraperitoneally into each animal at the time of operation. The male sex hormone was prepared from urine by a method modified from that of Dodds (1930).

To make sure that the hypophysis had been completely removed, the basisphenoid and the pituitary fossae of all the hypophysectomized animals were examined by serial section for pituitary remnants. If any pituitary tissue was demonstrable, the animal was discarded from the series.

The thyroid, suprarenal and thymus glands were not increased in weight by the injection of male sex hormone into hypophysectomized rats. In fact, the weights of these organs in the animals receiving the hormone, computed as percentages of the body weight, were slightly below those of the untreated hypophysectomized animals.

The thyroids of the hypophysectomized and also of the hypophysectomized animals that were treated showed low columnar epithelium, increase and hardening of the colloid. The suprarenals of both treated and untreated hypophysectomized animals showed atrophy of the cortex, with a more compact arrangement of the cortical cells.

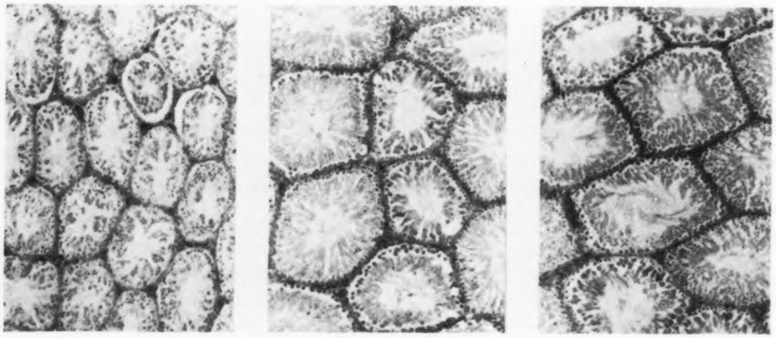
The effect of hypophysectomy and subsequent injection of male sex hormone on the percentages of body weight represented by formalin-fixed reproductive organs are shown in the table. The series is small, but the striking uniformity of the results give the findings significance. Hypophysectomy caused a considerable diminution in the weight of the testes, epididymis, prostate, and seminal vesicles. But when the animal received an injection of a preparation of male sex hormone (9 bird units dissolved in $\frac{1}{3}$ cc. of sesame oil) daily for about twenty days following hypophysectomy, the weights of these organs were approximately the same as those of the control group.

Histologically, the testis of the rat about twenty days after hypophysectomy shows an absence of spermatozoa and marked tubular degeneration with only spermatogonia and Sertoli cells remaining. The interstitial cells appear approximately normal. The epididymis, prostate and seminal vesicles show marked loss of secretion and atrophy of the mucous lining with some relative or actual increase of the supporting muscular and connective tissue (figs. 1 and 2).

The hypophysectomized animals treated with male sex hormone showed sex organs grossly and histologically not to be distinguished from the normal. Vatna's findings are thus confirmed but we have found, in addition, that the testes also were maintained normally as judged by histologic appearance. This finding is somewhat surprising in that it does not agree with the commonly accepted theories of testicular function.

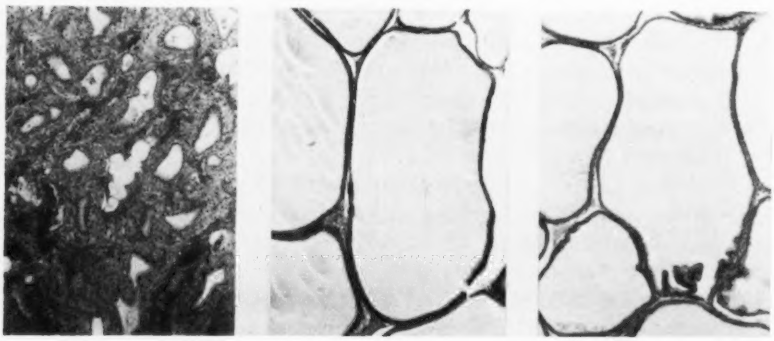
Obviously, the question arises as to whether the urinary extracts employed contained some physiologically active substance other than the male sex

hormone. The material injected has the following characteristics. It is thermostable; it is soluble in oil, benzene, alcohol and ether, and almost insoluble in water. Certainly none of the known pituitary hormones has these properties while testicular hormone does, and it seems improbable



A B C

Fig. 1. A. Section of testicle of hypophysectomized rat.
B. Section of testicle of normal rat used for control.
C. Section of testicle of hypophysectomized rat treated with male sex hormone.



A B C

Fig. 2. A. Section of prostate of hypophysectomized rat.
B. Section of prostate of normal rat used for control.
C. Section of prostate of hypophysectomized rat treated with male sex hormone.

that two different active physiologic substances would have so many properties in common. Moreover, it is not unreasonable to believe that the complete mechanism of fecundation is developed and maintained under the influence of one hormone, that from the interstitial cells of the testes.

Comparative weights of organs of rats

Calculated in percentage of body weight

	RAT	WEIGHT		DAYS	TESTES	EPIDIDYMIS	SEMINAL VESICLES	VENTRAL PROSTATE	DORSAL PROSTATE	THYROID	SUPRARENAL	THYMUS
		Original	Final									
Normal series	30N		224		1.050	0.286	0.065	0.072	0.020	0.014	0.008	0.048
	31C		192		1.228	0.390	0.422	0.230	0.081	0.046	0.012	0.091
	31CA		181		1.268	0.404	0.425	0.184	0.099	0.042	0.011	0.095
	41		303		0.795	0.276	0.187	0.146	0.032	0.009	0.007	0.058
	42A		185		1.113	0.356	0.248	0.148	0.051	0.021	0.011	0.091
	49		184		1.221	0.400	0.311	0.136	0.075	0.026	0.015	0.172
	57		176		0.940	0.349	0.342	0.161	0.053	0.007	0.010	
	59		114		1.205	0.185	0.133	0.068	0.019	0.012	0.015	0.053
	71B		127		1.330	0.364	0.120	0.145	0.034	0.010	0.014	0.112
	78C		239		1.063	0.371	0.366	0.172	0.055	0.010	0.010	0.113
Average weight (percentage of body)					1.116	0.338	0.262	0.146	0.052	0.020	0.011	0.092
Average weight (actual)			192		2.087	0.650	0.511	0.285	0.100	0.037	0.021	0.177
Hypophysectomized and treated with androtin	30I	280	231	20	0.953	0.288	0.150	0.063	0.038	0.006	0.005	0.048
	31I	166	128	23	1.387	0.512	0.530	0.188	0.082	0.014	0.007	0.064
	49A	150	122	28	1.314	0.412	0.415	0.168	0.058	0.016	0.004	0.078
	54	160	135	22	1.410	0.454	0.580	0.208	0.117	0.013	0.011	0.072
	59A	135	119	21	1.144	0.352	0.546	0.175	0.123	0.007	0.006	0.022
	65A	158	138	18	1.265	0.364	0.371	0.125	0.079	0.007	0.007	0.075
	67B	177	126	19	1.292	0.376	0.279	0.080	0.036		0.008	0.059
Average weight (percentage)					1.252	0.394	0.410	0.144	0.076	0.010	0.007	0.059
Average weight (actual)			143		1.746	0.547	0.547	0.194	0.104	0.015	0.010	0.085
Hypophysectomized	2	126	102	20	0.546	0.141	0.056	0.020	0.013		0.010	
	6	188	160	18	0.301	0.104	0.063	0.010		0.012	0.007	
	23	238	172	19	0.460	0.167	0.070	0.050	0.022	0.011	0.012	
	25	145	105	23	0.298	0.137	0.068	0.025	0.018	0.009	0.009	
	33	257	153	19	0.266	0.132	0.078	0.019	0.021	0.006	0.007	0.094
	44	221	121	18	0.476	0.223	0.088	0.039	0.016	0.008	0.013	0.022
	57B	203	164	21	0.338	0.135	0.053	0.020	0.015	0.008	0.004	0.066
	69A	238	150	20	0.527	0.155	0.065	0.021	0.015	0.009	0.007	0.082
	78	194	154	21	0.510	0.142	0.043	0.016	0.012	0.008	0.005	0.131
Average weight (percentage)					0.413	0.149	0.065	0.024	0.016	0.009	0.008	0.079
Average weight (actual)			142		0.586	0.209	0.092	0.036	0.024	0.013	0.012	0.120

SUMMARY

1. This study compares the findings in three series of rats; 1, normal; 2, hypophysectomized, and 3, those hypophysectomized and treated with male sex hormone from urine. The pituitary glands were removed by a method which combines features of the technic of Smith and of Thompson. Serial sections of the basisphenoid and pituitary fossae were made when the animal was killed to make certain that all pituitary tissue had been removed. The male sex hormone was prepared from urine by a method modified from that of Dodds.

2. The weights and histologic appearance of the thyroid, suprarenal and thymus glands in the hypophysectomized animals showed alterations from the normal, but there was no appreciable difference between these organs in the treated and untreated hypophysectomized rats.

3. In the untreated hypophysectomized animals, after twenty days, the weights of the testes, epididymis, prostate and seminal vesicles were decreased and microscopic examination showed considerable atrophy, but when the hypophysectomized animals received injections of male sex hormone over a similar period, the weights of all the sex organs were maintained and histologically the glands could not be distinguished from the normal.

REFERENCES

Dodds, E. C., A. W. Greenwood and E. J. Gallimore. 1930. Lancet, **1**, 683.

Freemantle, F. E. 1897. Guy's Hosp. Gaz. (London), **11**, 97, 119, 310.

McCullagh, D. R., W. K. Cuyler and J. T. Frawley. 1932. Trans. Roy. Soc. Canada, Section v, 183.

Moore, C. R. 1931. Proc. Second Int. Cong. Sex. Res. (London) 293; Journ. Amer. Med. Assn., **97**, 579.

Moore, C. R., W. Hughes and T. F. Gallagher. 1930. Amer. Journ. Anat., **45**, 109.

Moore, C. R. and L. C. McGee. 1928–1929. This Journal, **87**, 436.

Moore, C. R. and D. Price. 1932. Amer. Journ. Anat., **50**, 13.

Richter, C. P. and G. B. Wislocki. 1930. This Journal, **95**, 481.

Smith, P. E. 1930. Amer. Journ. Anat., **45**, 205.

Smith, P. E. and E. T. Engle. 1927–1928. Amer. Journ. Anat., **40**, 159.

Thompson, K. W. 1932. Endocrinol., **16**, 257.

Walsh, E. L., W. K. Cuyler and D. R. McCullagh. 1933. Proc. Soc. Exper. Biol. and Med., **30**, 848.

White, J. W. 1893. Ann. Surg., **18**, 152.

THE EFFECT OF VARYING LEVELS OF IODINE INTAKE ON THE THYREOGLOBULIN CONTENT OF THE THYROID GLAND[1]

MILDRED ELINOR JONES

From the Physiological Laboratory, University of Chicago

Received for publication October 9, 1933

Most investigators are agreed that inorganic iodine, fed or injected into an animal, in adequate amounts, for a sufficient length of time, increases the iodine content of the thyroid gland. This has been reported by Van Dyke (1), Krauss and Monroe (2), Evvard (3), Boruttau (4), Marine (5), Wheeler (6), Charvat (7), McClendon (8), and others, as a result of feeding KI to animals of various species. Contrary to these results Klein, Pfeiffer and Hermann (9), and Pfeiffer (10) did not find an increased iodine storage after KI administration to dogs. This discrepancy might be explained by differences of dosage and length of time of administration. Gray and Rabinovitch (11) in trying to correlate the degree of histological change with the size of the dose of KI, administered dosages of 0.0001, 0.001, 0.01, 0.05, and 0.1 gram per guinea pig, daily for fifteen to twenty days. The first dosage was ineffective, the second increased mitosis without otherwise producing an "active gland," and the last three caused histological changes typical of an "active gland" as well as a marked proliferation of epithelium. This mitosis was not proportional to the size of the doses. Gray, Haven and Loeb (12) found that feeding of KI produced no effect on the cells for thirty days, after which time the epithelium became lower and the acini enlarged. Van Dyke reported that when he fed one gram of KI daily to dogs for seventeen days, the total iodine content was increased but the ratio of cell iodine to total iodine was unchanged, whereas, when he injected 50 mgm. intravenously, if he removed the glands within sixty minutes, the total iodine was again high but the ratio of cell iodine to total iodine was very low. From the findings of these workers it would appear that Pfeiffer's dosage of only 3 mgm. daily and his time of administration of seven days were inadequate.

Although there has been considerable work done upon the histology of the gland and the storage of iodine, very little work has been done to determine the nature of the iodine compound stored. Marine and Rogoff

[1] This work was aided by the Biological Research Fund of the Rockefeller Foundation.

(14), after removing one thyroid from a number of dogs, injected 50 mgm. of KI intravenously, and then removed the second gland at intervals varying from a few seconds to thirty hours. The glands were compared for histological changes, iodine storage, and the activity of the substance stored as determined by the Gudernatsch test. Histological changes of an involutionary type were present in twenty hours. The storage of iodine was almost instantaneous whereas the elaboration of the hormone required a longer time, only a small fraction of the iodine taken up in a few seconds having been transformed into a specific hormone at the end of thirty hours. However, a slight difference in the activity between these glands and the controls was apparent in eight hours, and became more pronounced at the end of twenty hours. There was a great variation among dogs in the rate of formation of the active substance. This present investigation was carried out in order to determine a little more specifically the nature of the stored compound, and to see what effect large and small doses of KI would have upon the quantity stored.

Procedure. Since the method employed required about 10 grams of thyroid material, dogs, because of the size of their thyroids, were chosen as the most suitable experimental animals. To obtain this quantity, the right glands of ten dogs were compositely analyzed as the control and later, after iodine administration, the left glands were similarly analyzed. This procedure was adopted rather than using both glands from two series of five dogs each, where one series would serve as the control and the other for the experiment, because previous investigations had shown that the variation of the thyroid iodine content between different dogs was very large, whereas that between the two lobes of the same dog was small. The value found for the average difference between the two lobes of the same dog in a study of ten was 0.09 mgm. per gram of fresh gland, which is of the same order of magnitude as the value of 0.0055 mgm. per gram found by Rahe, Rogers, Fawcett and Beebe (15) for seven dogs, and that of 0.0156 mgm. per gram found by Watts (16) for ten dogs. In contrast to these slight differences are the variations between the different series of ten or more dogs, where it was found in twelve such studies that the maximal and minimal iodine contents were 1.138 mgm. per gram and 0.222 mgm. per gram, respectively. In spite of these large discrepancies in total iodine, the per cent precipitable by alcohol was always within the range of 94.6 per cent to 99.9 per cent. Accordingly then, after the right thyroid had been removed from six series of ten dogs each the first two series were fed 0.1 gram of KI per kilo. daily for six weeks, the next two 0.02 gram per kilo., and the last were kept as controls to see whether the removal of one gland led to a compensatory increase in the iodine content of the remaining gland. The low dose was that found by Gray and Rabinovitch (11) to produce minimal histological changes in the guinea pig thyroid, and the

high one was half of their maximal, as larger quantities proved too toxic to be tolerated by some dogs.

The method for extracting the iodine from the glands was that devised by Barnes (17) using one volume of 0.1 N sodium acetate per volume of glands. A preliminary investigation of dog thyroids had shown that from 97 per cent to 99 per cent of the total iodine could be removed in five extractions. The glands were, therefore, thoroughly ground in a mortar and extracted five times during a period of eight days. The iodine content of the extract was determined on duplicate 5 cc. samples by Kendall's (18) method, and the iodine in the gland residue was determined by the same method. It was then possible to calculate the average milligrams of iodine per gram of fresh gland. Barnes and Jones (19) had found that by using three volumes of 95 per cent alcohol, they could precipitate thyreoglobulin and possibly other related heavy molecules, leaving any lighter iodine-containing molecules such as thyroxin in solution. This procedure was applied to duplicate 5 cc. samples, after which they were centrifuged and the iodine content of the precipitate determined by Kendall's method.

Some of the extract was tested for biological activity by means of the Feather Germ test described by Juhn and Barnes (20).

Results. Owing to the loss of one or more dogs through snuffles, or the occurrence of an infected gland which had to be discarded, there were fewer dogs used in the analysis of the second gland than of the first in all groups except one. For this reason the weights are given in terms of average weight per gram of gland. The experiment for the groups marked A lasted six weeks while that of those marked B was terminated at the end of four weeks. The total milligrams of iodine per gram of gland were calculated from the total iodine extracted and the weight of the glands used. The extractions were always 98.0 per cent to 99.9 per cent complete. The results of the chemical analysis are shown in table 1.

The loss of iodine observed in control series A can not be explained; the gain in control series B is insignificant when compared to that of the KI fed groups. The amount of iodine stored was independent of the length of time of iodine administration, and of the dosages used but seemed to bear an inverse relation to the original iodine content of the gland. Most of the dogs showed no ill effects from the KI and were in good condition as judged by a study of body weights. There was a small loss of weight in some and a gain in others, but this was apparently not due to the KI administered for the same variations occurred in the control animals.

As judged from the Feather Germ test, the biological activity per unit of the iodine contained in the thyroid gland is about the same after iodine administration as before. In a series of twelve birds injected with control extract in doses containing from 0.386 to 0.084 mgm. of iodine, positive reactions were obtained for doses as low as 0.168 mgm. of iodine.

Thyroid extract after KI feeding, injected into six birds in doses from 0.706 to 0.176 mgm. of iodine caused pigment deposition in the feathers for all doses. Since the two series were injected at different times, to rule out any seasonal variation in the reactivity of the birds, thyroxin, the activity of which had been compared for all seasons, was injected into a similar series. This test while not absolutely quantitative showed the extract to be of the same order of activity per unit of iodine after iodine administration as before.

This work shows that KI in doses of 0.02 gram per kilo. daily for four weeks will produce as great storage of iodine as doses of 0.1 gram per kilo daily for six weeks. A certain minimal dose seems necessary, above which larger doses are superfluous. The Feather Germ test showed that this

TABLE 1

Showing the per cent of iodine increase and the per cent of iodine precipitable by 95 per cent alcohol

KI DOSAGE	NUMBER OF DOGS USED		AVERAGE WEIGHT PER GRAM GLAND		MGM. OF IODINE PER GRAM GLAND		PER CENT OF IODINE PRECIPITABLE BY 95 PER CENT ALCOHOL		PER CENT OF IODINE INCREASE
	Right	Left	Right	Left	Right	Left	Right	Left	
A. Control	10	9	1.240	1.411	0.836	0.558	96.6	99.4	−33.6
B. Control	10	10	0.922	0.940	1.155	1.272	98.4	97.8	10.13
A. 0.02 gm. KI	10	9	1.300	1.444	0.623	2.010	95.7	95.6	248.3
B. 0.02 gm. KI	9	7	1.333	1.230	0.660	2.046	96.3	94.9	210.0
A. 0.1 gm. KI	10	9	1.348	1.388	0.526	1.837	94.6	99.3	249.22
B. 0.1 gm. KI	10	8	0.920	1.012	1.085	2.668	99.9	95.8	146.8

increase in iodine was accompanied by an increase in biological activity, and the chemical analysis showed that the iodine was in a form precipitable by 95 per cent alcohol. While either thyroxin or thyreoglobulin might give a positive Feather Germ reaction, this could not have been thyroxin, since a thyroxin solution of equivalent iodine content added to thyroid extract before precipitation does not lead to any increase in the iodine of the precipitate. This points strongly to the fact that thyreoglobulin is the product elaborated and stored by the gland.

SUMMARY

1. Iodine in the form of KI fed to dogs produced an increase of 146.8 per cent to 249.22 per cent in thyroid iodine, 94 per cent to 99.9 per cent of which was in a form precipitable by 95 per cent alcohol, and therefore, probably thyreoglobulin.

2. Dosages of 0.02 gram per kilo. daily for four weeks were as effective in producing iodine storage as those of 0.1 gram daily for six weeks.

3. The biological activity per unit of iodine in the thyroid gland as found by the Feather Germ test is of the same order of magnitude after as before iodine administration.

4. The evidence is strongly in favor of an increased formation and storage of thyreoglobulin during KI administration, under the conditions of this experiment.

I wish to express my appreciation to Dr. A. J. Carlson and Dr. B. Barnes for their suggestions and assistance in carrying out this work.

REFERENCES

(1) Van Dyke, H. B. Journ. Biol. Chem., 1921, **45**, 325.
(2) Krauss, W. E. and C. F. Monroe. Journ. Biol. Chem., 1931, **89**, 581.
(3) Evvard, J. M. Endocrinol., 1929, **12**, 588.
(4) Borrutau, H. Zeitschr. exp. Path. u. Therap., 1916, **18**, 203.
(5) Marine, D. Publ. Health, Mich. St. Bd. Health.
(6) Wheeler, D. Can. Med. Assn. Journ., 1930, **22**, 157.
(7) Charvat, J. Endocrinol., 1931, **15**, 353.
(8) McClendon, J. F. Endocrinol., 1931, **15**, 281.
(9) Klein, W., G. Pfeiffer and G. Hermann. Biochem. Zeitschr., 1930, **225**, 344.
(10) Pfeiffer, G. Biochem. Zeitschr., 1929, **215**, 197.
(11) Gray, S. H. and J. Rabinovitch. Proc. Soc. exper. Biol. and Med., 1929, **26**, 468.
(12) Gray, S. H., F. L. Haven and L. Loeb. Proc. Soc. exper. Biol. and Med., 1927, **24**, 503.
(13) Van Dyke, H. B. Journ. Biochem., 1922, **54**, 11.
(14) Marine, D. and J. M. Rogoff. Journ. Pharm. Exp. Therap., 1916, 9.
(15) Rahe, J. M., J. Rogers, G. Fawcett and S. P. Beebe. This Journal, 1914, **34**, 72.
(16) Watts, C. F. This Journal, 1915, **38**, 356.
(17) Barnes, B. O. Proc. Soc. exper. Biol. and Med., 1932, **29**, 680.
(18) Kendall, E. C. Journ. Biol. Chem., 1914, **19**, 251.
(19) Barnes, B. O. and M. E. Jones. This Journal, 1933, **105**, 556.
(20) Juhn, M. and B. O. Barnes. This Journal, 1931, **98**, 463.

OBSERVATIONS ON DIRECT INTRA-ARTERIAL DETERMINATION OF BLOOD PRESSURE IN TRAINED UNANESTHETIZED DOGS[1]

WILLIAM M. PARKINS

From the Biological Laboratory, Princeton University

Received for publication October 3, 1933

Recently Dameshek and Loman (1932) described an instrument for the direct determination of intra-arterial pressure in man which appeared to be eminently suitable for the determination of mean arterial pressure in the trained unanesthetized normal and bilaterally adrenalectomized dog. The present study is concerned with the application of the Dameshek and Loman instrument to the dog subjected to various conditions of laboratory experimentation. A detailed description of the instrument and its use is given in the above cited reference and need not be repeated here.

Sixty-six dogs, male and female, weighing from 8 to 15 kgm. were used in this study. The dogs were trained to lie on their backs, completely relaxed. The femoral artery on the central medial surface of the proximal end of the thigh is easily accessible for palpation and puncture. Pressure readings were taken when equilibrium between the blood stream and the manometer had been reached and maintained for several seconds, and only when free pulsations indicating a center puncture and free flow through the needle were readily observed. Cannulation with a three way glass cannula connected to a standard U type mercury manometer apparatus was used as a standard for comparative readings.

RESULTS. 1. *Comparison of needle-puncture Tycos sphygmomanometer with cannulation mercury manometer recordings.* Table 1 shows one of the more complete experiments in this group of ten animals where a wide range of pressure levels was obtained and simultaneous readings made by two individuals.

A trained male dog, weighing 10 kgm., was placed on the animal board and a normal resting pressure reading was taken by the needle puncture method, previous to the administration of ether. After etherization to the point of disappearance of the eye reflex, the left femoral artery was quickly cannulated with a large three-way glass cannula and connected

[1] These investigations have been aided by a grant from the Josiah Macy, Jr. Foundation, New York.

with the mercury manometer. A second needle puncture was made through the skin and into the right femoral artery. Several minutes of continuous readings were recorded simultaneously from the two methods. The needle and stopcock were cleaned and a third puncture was made just previous to the very slow 1 cc. injections of morphine sulphate into the left jugular vein. Simultaneous readings were again taken, while the mercury manometer readings were recorded on the kymograph.

TABLE 1

Comparison of blood pressure readings taken simultaneously by two methods

TIME	CANNULA MERCURY MANOMETER	NEEDLE SPHYGMO-MANOMETER	ERROR	REMARKS
2:45		104		Unanesthetized
3:08	133	131	−2	Ether anesthesia
3:12	137	136	−1	
3:15	134	133	−1	
3:20	139	137	−2	Morphine—3:23
3:24	121	120	−1	Morphine—3:25
3:27	98	98	0	
3:30	93	94	+1	Morphine—3:31
3:34	80	83	+3	
3:40	83	85	+2	Adrenaline
3:46	112	111	−1	Adrenaline
3:49	138	135	−3	Adrenaline
3:50	142	142	0	Adrenaline
3:52	180	177	−3	
4:03	137	138	+1	
4:08	92	94	+2	Histamine
4:10	63	63	0	
4:12	36	39	+3	
4:15	58	56	−2	
4:18	78	78	0	
4:25	80	81	+1	Exsanguination
4:36	56	56	0	
4:40	24	27	+3	Death

Adrenaline, 1:100,000, was slowly injected into the jugular vein to raise the blood pressure to an abnormally high level. Histamine, 1:100,000, was given to lower the blood pressure. Exsanguination from the carotid artery ended the experiment with the blood pressure readings at the death level. The maximum error of the comparative readings for this series was 4 mm. Hg; for this particular experiment 3 mm. Hg. The average of errors is +2 and −1.7. The close parallelism of the two groups of readings supports the accuracy of the Dameshek and Loman instrument for most practical purposes where a mean arterial pressure determination is sufficient.

In four of these experiments needles varying from 18 to 26 gauge were used to test the influence of the damping effect produced by the needle upon maximum readings. A conclusion was drawn, similar to that of Dameshek and Loman from tests on an artificial circulation machine, namely, the finer the needle the greater the effect upon the fluctuations about the mean pressure, with only insignificant variations in the actual maximum readings obtained. A 22 gauge needle has been found preferable.

In other experiments the mercury manometer was substituted for the Tycos sphygmomanometer in the Dameshek and Loman apparatus. It

TABLE 2

Individual variations in mean arterial pressure (Dameshek and Loman instrument) over periods of days

DOG 121					DOG 88		
Date	Pulse	Mean blood pressure	Pulse	Mean blood pressure	Date	Pulse	Mean blood pressure
	Initial determination		Check determination				
June 8	90	112	94	115	Feb. 6	88	104
8	96	114	88	116	8	84	100
9	80	113	86	111	10	84	103
9	88	115	88	114	13	72	98
10	76	110	84	112	16	76	101
11	82	113	90	116	17	72	98
11	92	115	80	110	20	80	102
12	88	112	90	112	23	80	100
12	98	116	88	112	24	76	98
15	88	114	92	115	24	86	104
15	80	112	88	114	28	90	104
16	72	106	70	107	March 2	84	105
17	68	105	80	110	4	80	103
17	76	107	80	111	6	80	96
18	72	108	76	110			
19	80	112	80	110			
20	84	113	88	115			

was found that the mercury columns completely damped out the slight fluctuations which escaped the 22-gauge needle and practically a straight line was recorded on the smoked drum of the kymograph. However, the maximum readings were found to be practically the same in both manometers. It should be kept in mind that allowance must be made for the intrinsic lag of the system when rapid changes in pressure are recorded.

2. *Normal blood pressure of trained unanesthetized dogs.* Data on direct intra-arterial blood pressure of trained unanesthetized dogs are comparatively rare. Most of the values given for the unanesthetized dogs have been obtained by modification of the indirect Riva Rocci cuff method.

Over 1200 pressure readings have been taken in this laboratory by means of the Dameshek and Loman instrument, all the normals of which fall within the general range and about the average presented in the following two tables.

Table 2 shows the findings in two typical experiments. In one case (dog 121) pressure readings were taken in the morning before feeding and again late in the afternoon. This routine was carried out over a period of thirteen days. In the other case (dog 88) pressure determinations were taken before feeding but, at more or less irregular intervals, over a period of one month. Check determinations were taken on dog 121 at approximately the same time or immediately following the initial reading. The pulse rate was recorded as a rough index, used in combination with external criteria, to judge the degree of rest, relaxation, etc., of the animal at the time the readings were taken.

It is noticeable that the maximum variation of blood pressure in dog 121 was 11 mm. Hg in the initial group, and 9 mm. Hg in the check group. The average for the initial readings was 111.5 against 112.3 in the check series. The maximum variation for dog 88 was 9 mm. Hg occurring in a four day interval. The training of the animal for constant conditions of rest, relaxation, and freedom from emotional states is extremely important in order to obtain comparable accurate checks.

Table 3 is a summary of the average reading taken on 35 well trained normal dogs and 31 bilaterally adrenalectomized dogs maintained in normal physiological conditions by injections of adrenal cortical hormone, upon which regular pulse and pressure determinations were taken over periods ranging from days to months. The average of the readings for each dog was taken for this table. The range of blood-pressure values is from 92 to 120 mm. Hg in the normal and 93 to 116 in the adrenalectomized group. The average for each group is respectively 107.2 and 106.1.

This table clearly shows that on maintenance doses of cortical hormone the blood pressure of the bilaterally adrenalectomized dog is normal.

The individual variations, as shown in figure 2, are considerably less than those reported by Allen (1923), Kolls and Cash (1923), Petroff (1929) and Ferris and Hynes (1930) which were obtained by quite different methods and conditions. The values given in table 3 are in rather close agreement with results of other investigators, particularly those of Allen (1923) which were obtained by a modified Rica Rocci cuff method.

3. *Effect of anesthetics on blood pressure.* In certain experiments, particularly those where trauma is produced, an anesthetic is obviously necessary.

Experiments were carried out by the use of the needle-puncture sphygmomanometer method, to determine the changes in intra-arterial blood pressure upon administration of and recovery from certain drugs and

anesthetics. These particular ones were chosen because of their use in this laboratory, and elsewhere, for anesthetic purposes. These data have been useful to us and may be of interest to others.

TABLE 3

Mean arterial blood pressure values for trained unanesthetized dogs

NORMAL UNOPERATED DOGS			BILATERALLY ADRENALECTOMIZED, MAINTAINED IN NORMAL PHYSIOLOGICAL CONDITIONS BY INJECTION OF ADRENAL CORTICAL HORMONE		
Dog number	Pulse rate per minute	Mean blood pressure	Dog number	Pulse rate per minute	Mean blood pressure
61	100	106	*61	96	110
62	92	110	*62	98	116
68	102	111	*68	106	112
69	104	115	*69	100	112
71	78	108	*71	70	112
75	98	106	*75	88	104
67	72	101	45	82	93
70	100	99	47	74	112
72	90	94	48	100	106
73	102	92	50	88	110
74	86	112	51	100	97
76	100	102	52	96	105
77	90	111	53	88	104
78	100	110	55	92	103
79	84	112	65	102	112
80	80	94	66	80	103
81	84	104	20	74	105
82	100	112	12	60	112
83	76	108	84	88	111
86	98	109	91	100	104
88	80	103	92	72	109
90	96	120	*88	72	101
97	76	108	93	70	97
96	84	112	94	72	93
98	80	110	103	88	99
99	60	106	111	96	104
101	60	120	114	80	108
104	88	108	*107	70	108
106	72	99	*120	68	110
107	90	112	122	90	116
115	88	112	127	80	104
120	92	104			
121	60	112			
123	68	104			
125	72	106			

* Indicates duplication in the two series.

The pressure and pulse observations in figures 1 and 2 were taken at an interval of 45 minutes following subcutaneous injections. Where intra-

venous injections were employed, readings were taken at regular intervals from the time of the injection until the end of the experiment, and the value of the maximum effects recorded. Determinations were made in the ether experiments at the point of disappearance of the eye reflex. When constant prolonged ether anesthesia was necessary, using the closed system (Livingston and Hrdina, 1930), the same depth as used for the more temporary experiments was carried out as nearly as possible. Atropine sulphate was given in a 0.01 per cent solution subcutaneously or intravenously, depending upon the nature of the experiment. Morphine sulphate, 5 to 10 mgm. per kilo in a 1 per cent solution was given subcutaneously or intravenously.

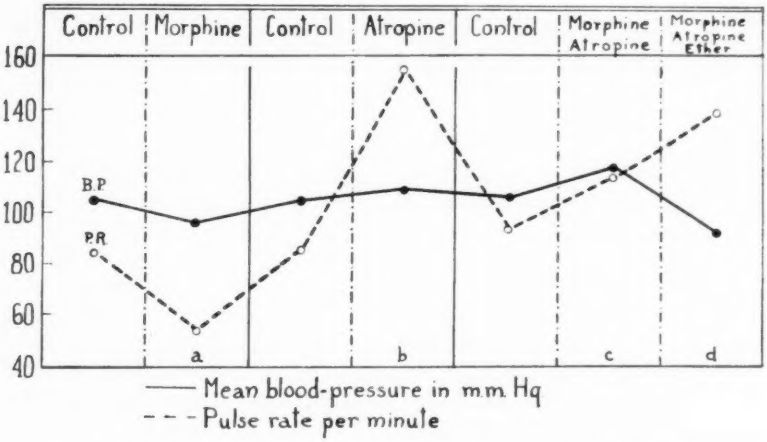


Fig. 1. Effect of various drugs on mean arterial pressure and pulse rate.

Figures 1 and 2 are graphic presentations summarizing the results of 70 experiments. Eight to 12 different animals were used in each group where individual drugs and combinations were studied. Each point plotted represents the average for 10 experiments in each respective group.

As presented in figure 1, *a* and *b*, both morphine and atropine when independently administered produce a marked effect on pulse rate, which presumably is due to their action on the parasympathetic control of the heart. When a simultaneous injection of both drugs is made, the action of morphine in slowing the pulse rate is abolished. However, the antagonism to the paralysis by atropine is clearly shown in figure 1, *c*.

The change in blood pressure is very slight indeed with both morphine and atropine. This change however, as might be predicted, follows the change in pulse rate. A slight decrease occurred in the experiment with morphine and an increase in most cases where atropine was injected sub-

cutaneously. When atropine was injected intravenously the usual temporary increase in blood pressure was recorded. This effect was not present in cases of slow subcutaneous absorption.

When ether was administered to an animal previously injected with morphine and atropine, there was a consistent progressive decrease in blood pressure, in spite of an increase in pulse rate of a similar degree, as shown in figure 1, *d* and figure 2, *d*.

Figure 2, *b* and *d*, shows a rather drastic decrease in blood pressure where ether and morphine were in combination. The presence of atropine had little effect on the reaction of blood pressure to ether anesthesia. (Compare *a* and *c* of fig. 2.) When ether is administered to a normal untreated

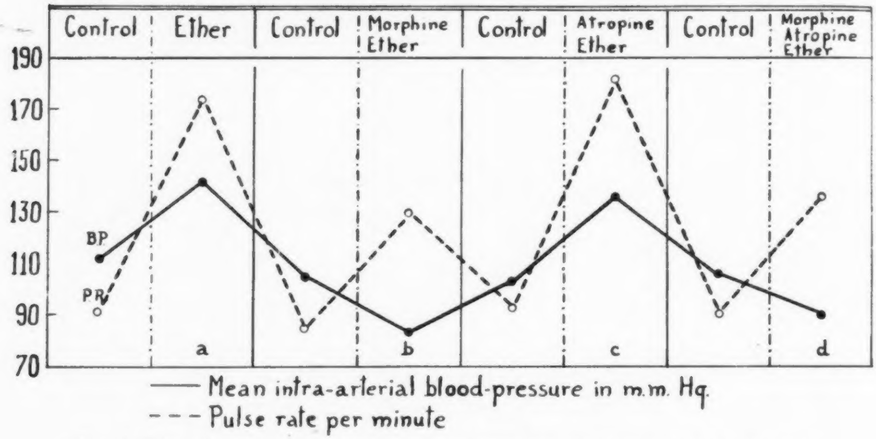


Fig. 2. The effect of various anesthetics on mean arterial pressure and pulse rate.

or to an atropinized animal, although great individual variations were found, the average shows a drastic increase in pressure.

The point of particular interest here is the effect of morphine on the response of arterial blood pressure to ether anesthesia. Out of 25 experiments where these two drugs were used in combination, there was but one exception to a marked decrease in mean arterial blood pressure, and in this case there was no change.

These data on the morphine-ether combinations are not in agreement with findings reported by Blalock (1928), where ether was administered to dogs previously narcotized with morphine. Blalock reported an increased pressure under these conditions. This point was put to further tests by reversing the order and changing the method of administration. Morphine was injected intravenously into an animal under constant ether anesthesia. Again there was a constant gradual decrease in pressure which

followed simultaneously with the slow injection of morphine. Table 1 shows a typical example. This effect was found to be present regardless of the depth of ether anesthesia. It is quite clear that the effect of a substance administered to an etherized animal may, in some cases, be quite different, possibly reversed from the effect in the normal unanesthet zed condition.

The explanation of the rather surprising effect of the ether morphine combination is not at present clear. It did not seem wise, at present, to be drawn aside from the original purpose of this study to a time consuming pharmacological investigation, in order to review, discuss, and attempt to explain the mechanisms involved in the response of arterial blood pressure to the drugs used.

The writer wishes to acknowledge his indebtedness to Dr. W. W. Swingle for suggesting the problem, and for his interest and helpful criticism throughout the investigation.

SUMMARY AND CONCLUSIONS

1. The instrument described by Dameshek and Loman (1932) has been found applicable to great advantage in the trained unanesthetized dog, particularly in following the mean intra-arterial blood pressure at intervals over a relatively long period of time.
2. The accuracy of the values obtained by the use of this instrument in the dog has been determined from a large number of comparative readings taken over complete ranges of high, low, and normal blood pressure levels.
3. The mean blood pressure of the normal well trained unanesthetized dog tends to remain constant over a considerable period of time.
4. The average of blood pressure values for 57 normal well trained unanesthetized dogs under constant controlled conditions was 106 mm. Hg. The range of readings for 66 individuals was from 92 to 120 mm. Hg.
5. The blood pressure of the bilaterally adrenalectomized dog on maintenance doses of cortical hormone is normal.
6. The effects upon intra-arterial blood pressure of certain drugs used in this laboratory primarily for anesthetic purposes are briefly summarized.

REFERENCES

Allen, F. M. 1923. Journ. Met. Res., **4,** 431.
Blalock, A. 1928. Surg., Gyn. and Obst., **46,** 72.
Dameshek, W. and J. Loman. 1932. This Journal, **101,** 140.
Ferris, H. W. and J. F. Hynes. 1930–31. Journ. Lab. Clin. Med., **16,** 597.
Kolls, A. C. and J. R. Cash. 1923. Johns Hopkins Hosp. Bull., **34,** 49.
Livingston, H. M. and L. S. Hrdina. 1930. Journ. Lab. Clin. Med., **16,** 74 (fig. 3).
Petroff, J. R. 1929–30. Pflüger's Arch., **223,** 477.

PILOCARPINE AND INSULIN SECRETION

SAMUEL APPELROT

From the Department of Physiology of the American University of Beirut, Beirut, Syria

Received for publication October 9, 1933

The effect of pilocarpine on blood sugar in animals has been studied by many investigators and the consensus of opinion is that large doses of pilocarpine produce hyperglycemia.

In the light of La Barre's work (1927a) and the recent findings by Kuré and his associates (1933), which seem to show that stimulation of the parasympathetic nerves causes an increased insulin secretion, one would expect that pilocarpine, which is considered as a parasympathetic stimulant should also produce hypoglycemia instead of the hyperglycemia that has been so widely reported.

One reason for this unexpected result can be found in the early reports by Dale and Laidlaw (1912) and Jackson (1913) who have found that pilocarpine stimulates the adrenals to secrete adrenalin. More recent work by LeGrand and Bierent (1927a), Viale and Napoleoni (1929) and Inouye (1931) have confirmed the earlier reports that pilocarpine stimulates the adrenals through the splanchnic nerves (Papilian and Jianu, 1929) to a greater production of adrenalin. LeGrand and Bierent (1927b) assume that in addition to the increased production of adrenalin there exists also an increased thyroxin secretion after the administration of pilocarpine, because in their experiments after a double adrenalectomy the pilocarpine hyperglycemia that was markedly reduced has completely disappeared after thyroidectomy.

Such an increased output of adrenalin and thyroxin may account in part for hyperglycemia due to pilocarpine.

Another possible explanation for pilocarpine hyperglycemia may be found in the concurrent concentration of the circulating blood by the loss of fluids, under the influence of pilocarpine, which may amount to about 10 per cent to 40 per cent of the total volume (Bornstein and Vogel, 1921).

This work was undertaken to investigate the part played by the internal secretion of the pancreas in the complex mechanism that is responsible for the pilocarpine effect on blood sugar.

METHOD. Blood sugar determinations were made by the Benedict method (1931) on twelve normal and twelve pancreatectomized dogs before and after intramuscular injections of a 10 per cent solution of pilocarpine hydrochloride in distilled water. Six animals of each group were

given a dose of 0.5 mgm. of pilocarpine per kilo body weight and the other six were injected with 1 mgm. per kilo body weight. Blood samples, drawn directly from the heart, were taken once before the administration of the drug and five more times at half an hour intervals after the injection of pilocarpine. The collection of samples was started regularly, early in the morning between 7:00 and 7:30 a.m., with the animals in a fasting state, their last meal having been given to them eighteen hours previously. As controls, six normal dogs and six pancreatectomized dogs were either injected with distilled water instead of pilocarpine or the injection was entirely omitted and blood sugar estimations were made in exactly the same manner. To ensure uniformity in the degree of excitement of the various animals, they were all trained to lie attached quietly on their backs to animal boards during a period of three and one-half hours.

Results. The injection of 0.5 mgm. of pilocarpine per kilo body weight to normal dogs gave rise to a slight elevation of blood sugar in 4 animals, varying from 4 to 24.6 mgm. per cent, and to a fall in blood sugar in 2 animals, varying from 12.4 to 15 mgm. per cent, with a total average rise of 11.6 mgm. per cent. The administration of 1 mgm. of pilocarpine per kilo body weight to normal dogs results in a consistent rise in blood sugar in all animals, the average being 35.34 mgm. per cent.

In the pancreatectomized animals the injection of 0.5 mgm. of pilocarpine per kilo body weight results in a very pronounced rise over and above the fasting blood sugar in all animals, consisting of an average rise of 120.8 mgm. per cent. The administration of 1.0 mgm. of pilocarpine per kilo body weight gives rise to an average elevation of 80.06 mgm. per cent. In the normal controls rise of an average of 11.6 mgm. per cent was noted, while in the pancreatectomized animals the average rise is 37 mgm. per cent.

Discussion. The outstanding feature of the results is the difference in response of the normal and the pancreatectomized dogs. The reason for this difference probably lies in the fact that pilocarpine influences the existing blood sugar by at least three different mechanisms. Two of the mechanisms, namely, the blood concentration effect and the adrenalo-thyroid stimulation, bring about an elevation in the blood sugar. The third mechanism consists probably of the parasympathetic stimulation of the islet tissue of the pancreas resulting in an increased production of insulin which tends to lower the blood sugar. The sum total of these three effects, the last being antagonistic to the former two, gives rise in the normal animals to a slight hyperglycemia because the first two mechanisms overbalance the third. With higher doses of pilocarpine, the adrenals, (the thyroids?), and the water excretory organs are probably stimulated more intensely than the islets, giving rise consistently to a hyperglycemia.

In the pancreatectomized animals, the adrenalo-thyroid mechanism is free to act, rise in blood sugar is accordingly higher and consistent.

These data lead us therefore to conclude that in the complex mechanism producing pilocarpine hyperglycemia, the pancreatic internal secretion is stimulated to a greater production of insulin, which with the high doses employed in this work was overbalanced by other antagonistic influences. Further evidence to support this conclusion is supplied by Suematsu (1930), who reports that under the influence of pilocarpine a hyperplasia of the islands of Langerhans is noted.

Pilocarpine can therefore still be considered as a parasympathetic stimulant producing an increased insulin production in the same way as does the stimulation of the parasympathetic nerves.

SUMMARY

Large doses of pilocarpine produce in normal dogs a slight rise, and in pancreatectomized dogs a very pronounced rise in blood sugar. The mechanisms responsible for the hyperglycemia and the reasons for the difference in response to pilocarpine by the two groups of animals is discussed and the conclusion drawn that pilocarpine stimulates the islet tissue of the pancreas, but that this effect is overshadowed by other antagonistic mechanisms such as stimulation of the adrenal and possibly the thyroid glands.

REFERENCES

Benedict, S. R. 1931. Journ. Biol. Chem., **92**, 141.
Bornstein, A. and R. Vogel. 1921. Biochem. Zeitschr., **118**, 1.
Dale, H. H. and P. P. Laidlaw. 1912. Journ. Physiol., **45**, 1.
Inouye, T. 1931. Journ. Biochem., **14**, 215.
Jackson, D. E. 1913. Journ. Pharm. Exp. Therap., **4**, 291.
Kuré, K., Tatsuo Tanishina and Shigeo Okinaka. 1933. Quart. Journ. Exp. Physiol., **22**, 329.
LaBarre, J. 1927a. Compt. rend. Soc. Biol., **96**, 193.
1927b. Arch. Internat. d. Physiol., **29**, 227.
LeGrand, A. and G. Bierent. 1927a. Compt. rend. Soc. Biol., **96**, 291.
1927b. Compt. rend. Soc. Biol., **97**, 1483.
Papilian, V. and M. Jianu. 1929. Compt. rend. Soc. Biol., **100**, 379.
Suematsu, T. 1930. Fol. Endocrinol. Japon., General Summary, **6**, 45.
Viale, G. and L. Napoleoni. 1928. Compt. rend. Soc. Biol., **99**, 2006.